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References must include the full titles of all articles cited. Journal abbreviations should comply with the conventions of the latest edition of the World List of Scientific Periodicals (1965), *e.g.*

JANOTA, I. (1967) Adnexal Skin Tumours in Nigeria. *Br. J. Derm.*, **79**, 259.

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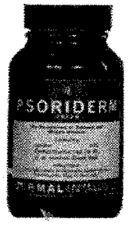

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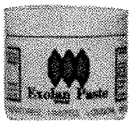

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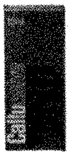

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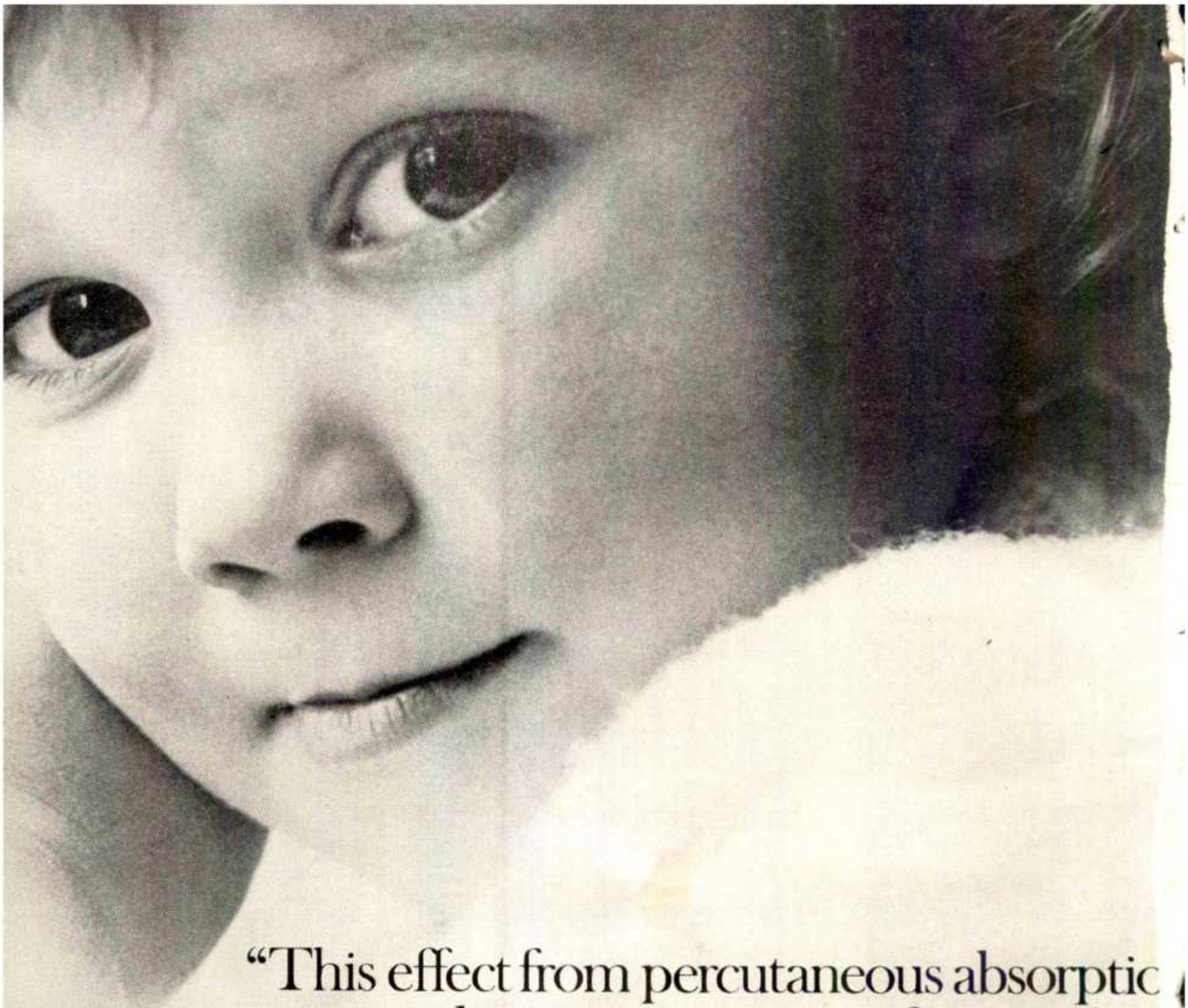

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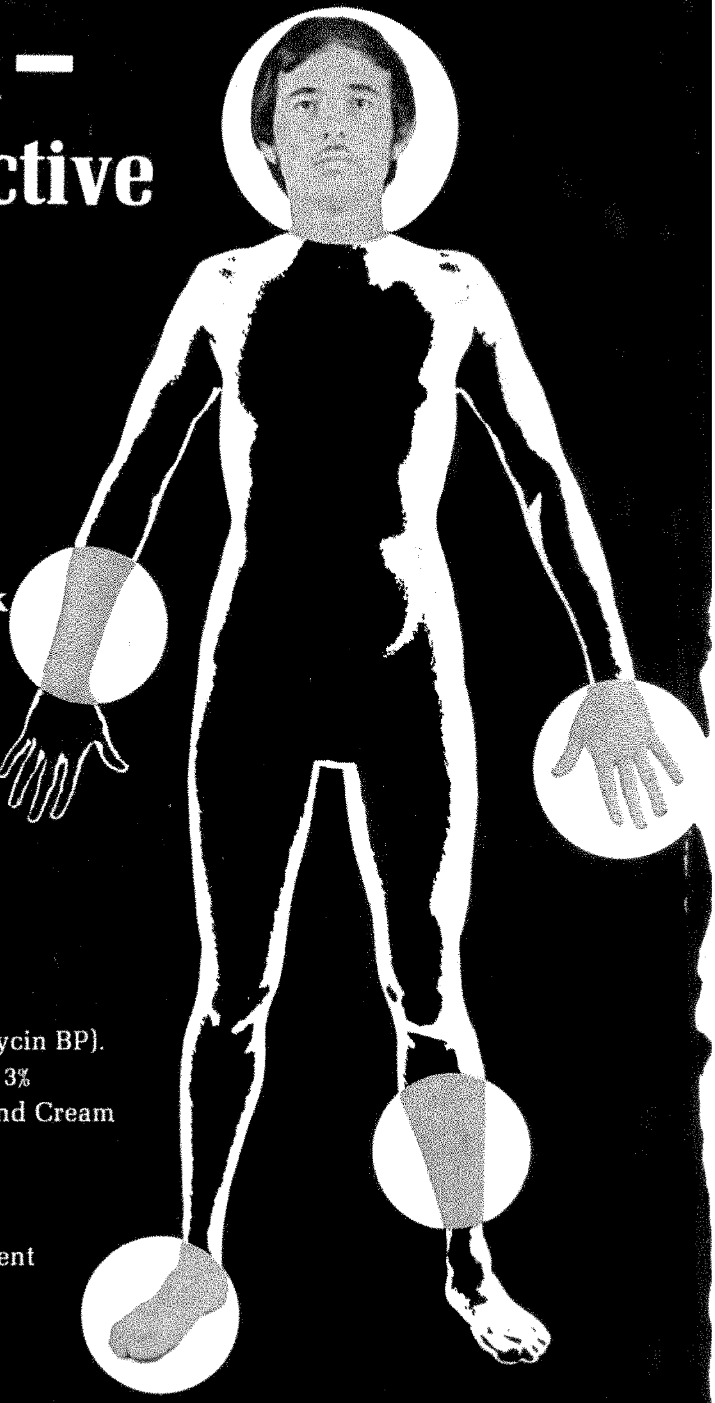

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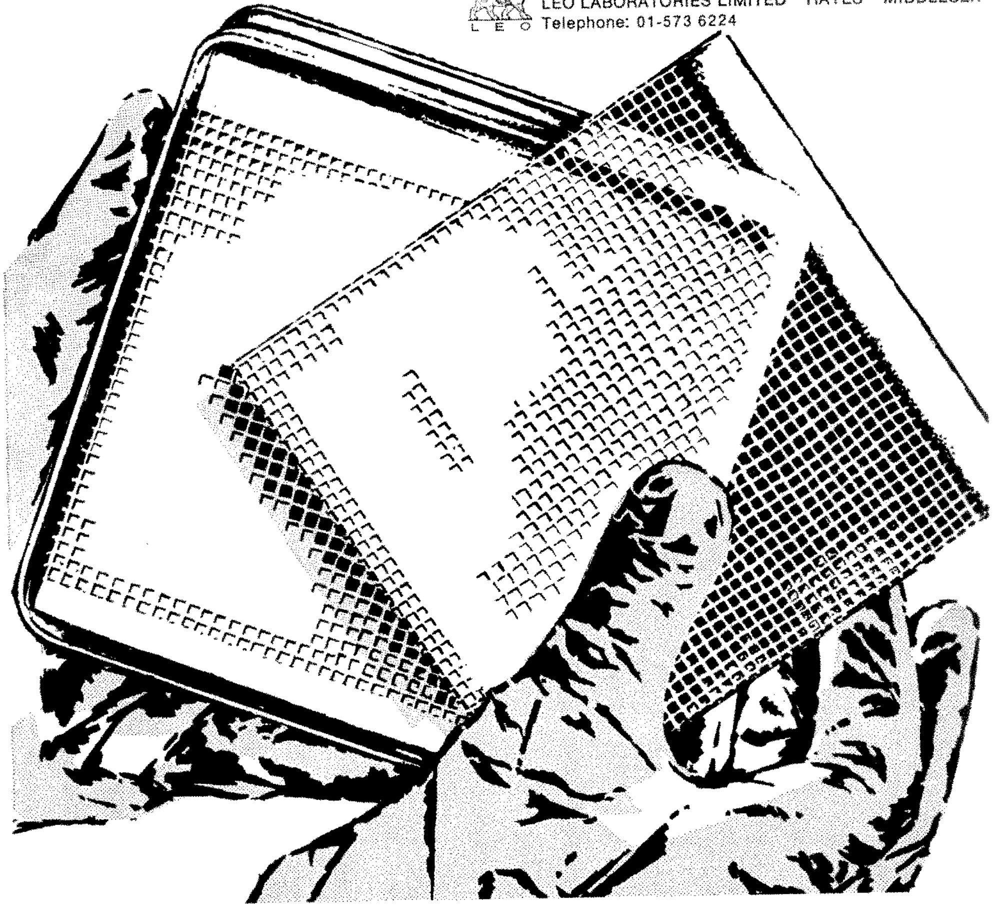

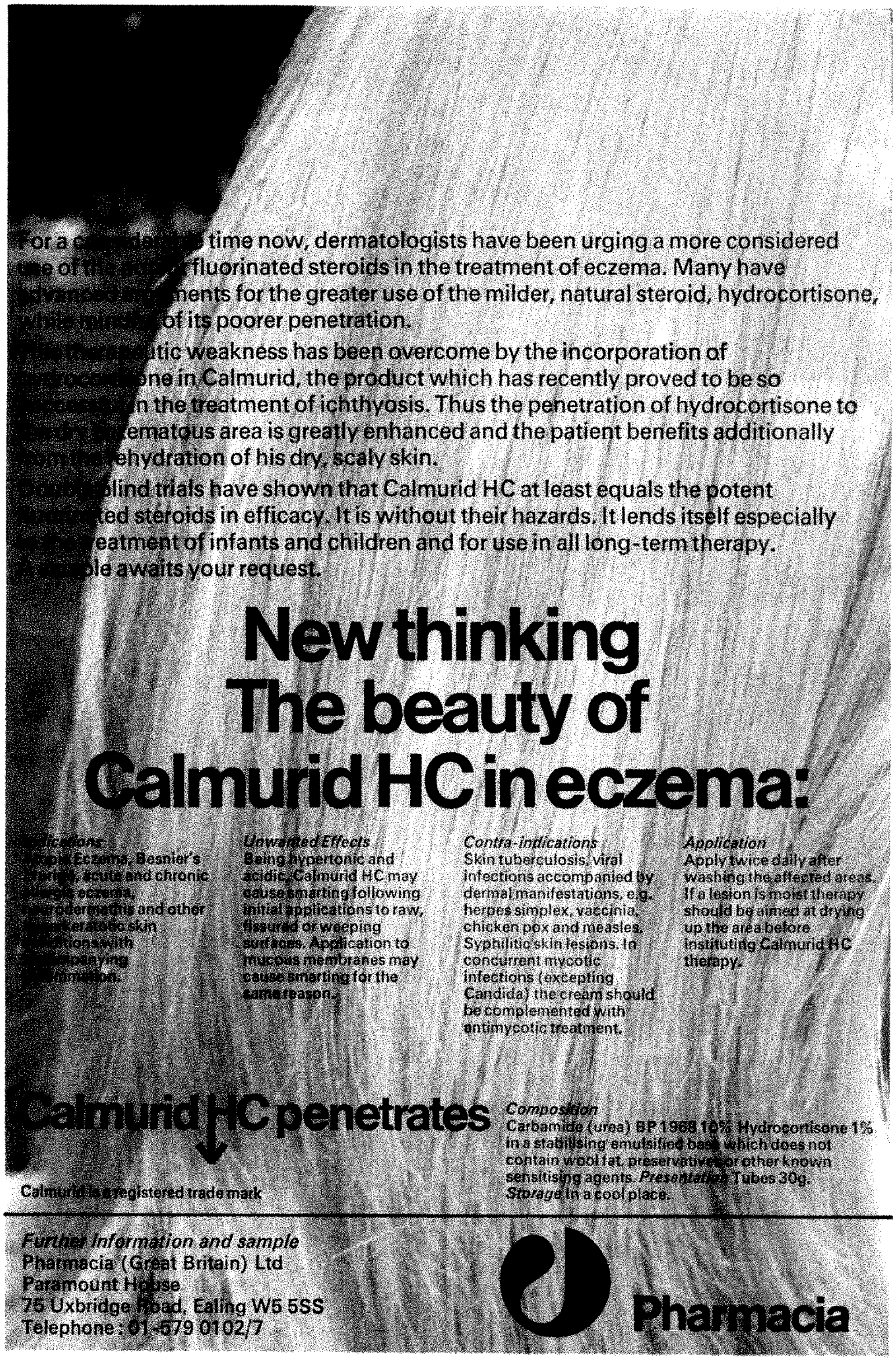

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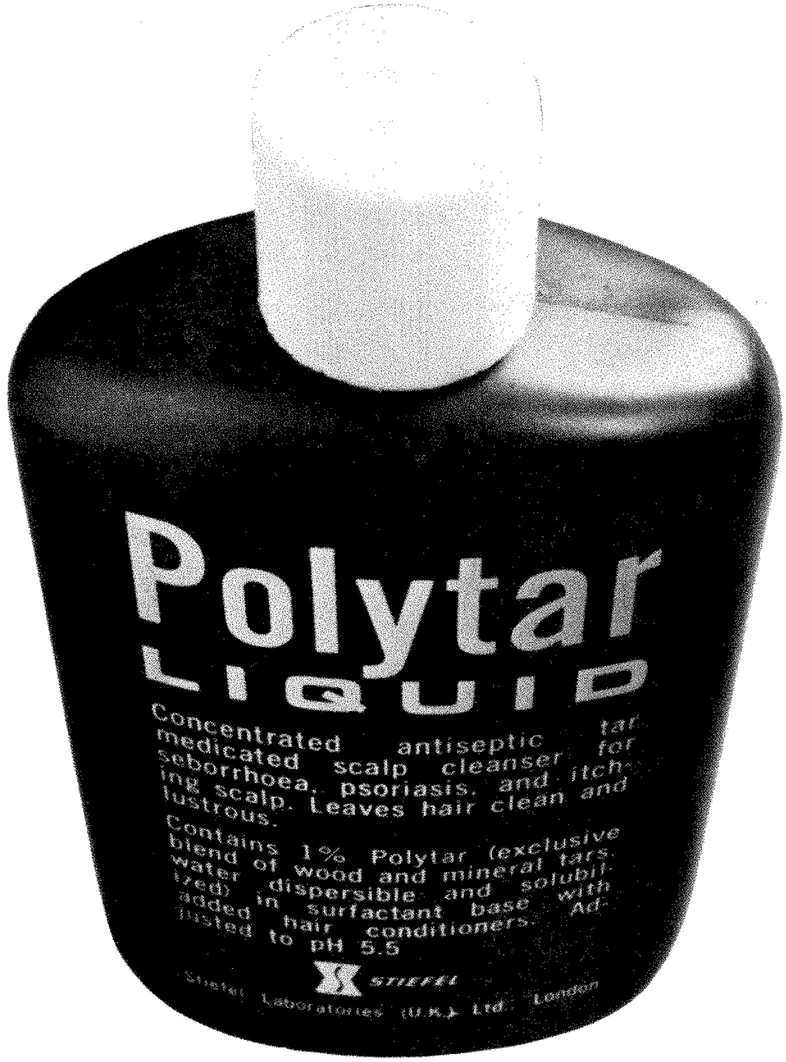
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CONTENTS

No. 1.—JANUARY

PAGE

Clinical and Laboratory Investigations

A SURVEY OF ICHTHYOSIS VULGARIS IN ISRAEL. L. Ziprkowski and A. Feinstein . . . 1
DISSEMINATED ELASTOSIS PERFORANS SERPIGINOSA IN FOUR MONGOLOIDS. RECOGNITION OF RESIDUAL CHANGES. James E. Rasmussen . . . 9
EPIDERMAL "TURNOVER TIME"—A RE-EXAMINATION. Kenneth M. Halprin . 14
HISTOCHEMICAL APPLICATIONS OF SKIN SURFACE BIOPSY. R. Marks . . 20
ULTRASTRUCTURE OF ADULT EPIDERMAL CELLS IN MONOLAYER CULTURE. Hilary Constable . . . 27
EXPERIMENTAL ZOSTERIFORM HERPES SIMPLEX VIRUS INFECTION IN MOUSE SKIN. T. W. E. Robinson and J. R. Dover . . . 40
THE CONTROL OF GLYCOGEN UTILIZATION IN SKIN; PHOSPHORYLASE *B* KINASE AND PHOSPHORYLASE *A* PHOSPHATASE. P. D. Mier and A. H. M. Sutorius 49

Pharmacology and Treatment

TREATMENT OF VITILIGO WITH ORAL 4,5′,8-TRIMETHYLPSORALEN (TRISORALEN) S. S. Bleehen . . . 54
THE EFFECT OF PROLONGED TOPICAL APPLICATION OF AN ANTICHOLINERGIC AGENT ON THE SEBACEOUS GLANDS. M. Cartlidge, J. L. Burton and S. Shuster . . . 61
EFFECT OF TOPICAL TETRAYNOIC ACID ON SEBUM EXCRETION. T. S. Haroon, J. Hall and A. Haroon . . . 64
THE EFFECT OF TOPICAL TETRAYNOIC ACID ON THE SEBORRHOEA OF ACNE. J. L. Burton and Sam Shuster . . . 66
A STUDY OF THE EFFECT OF PREDNISONE AND AN ANTIHISTAMINE ON PATCH TEST REACTIONS. E. Feuerman and A. Levy . . . 68
TREATMENT OF IDIOPATHIC HYPERHIDROSIS WITH IONTOPHORESIS OF TAP WATER AND POLDINE METHOSULPHATE. Katherine Grice, H. Sattar and Harvey Baker . . . 72
TREATMENT OF PSORIATIC NAIL DYSTROPHY. E. Abell . . . 79
PENETRATION AND DOSE OF INJECTIONS WITH THE PORTON JET INJECTOR. P. Gaylarde, A. L. Macmillan and I. Sarkany . . . 83

Comment

ANTIMETABOLITES AND PSORIASIS. Peter C. H. Newbold . . . 87
Henry Sermon . . . 90

Case Reports

ACROKERATOSIS VERRUCIFORMIS OF HOPF WITH STEATOCYSTOMA MULTIPLEX AND HYPERTROPHIC LICHEN PLANUS. Julian Verbov . . . 91
ECCRINE SWEAT GLAND CARCINOMA WITH METASTASES. Vinay K. Dave . . 95

Society Proceedings

ROYAL SOCIETY OF MEDICINE, MAY 20TH AND JUNE 17TH 1971 . . . 98

Correspondence

PSORIASIS AND HYDROXYUREA. R. Touraine, J. Revuz and M. Tulliez . . 102
"LYMPHOCYTE TRANSFORMATION". Suzanne Alexander . . . 103

No. 1.—JANUARY—*cont.*

PAGE

Books

COMMON SKIN DISEASES (BEHRMAN, LABOW AND ROZEN) 103

Quarterly Review

CONTACT DERMATITIS XVII. REACTIONS TO CONTACT ALLERGENS GIVEN ORALLY OR SYSTEMICALLY. Etain Cronin 104

News and Notices 108

No. 2—FEBRUARY

Clinical and Laboratory Investigations

FACTORS CONTRIBUTING TO OCCASIONAL FAILURES IN THE LABORATORY DIAGNOSIS OF BULLOUS PEMPHIGOID BY INDIRECT IMMUNOFLUORESCENCE. Tadeusz P. Chorpelski and Ernst H. Beutner 111

DEMONSTRATION OF INTERCELLULAR IMMUNOFLUROESCENCE AND EPIDERMAL HYSTERESIS IN BULLOUS FIXED DRUG ERUPTION DUE TO PHENOLPHTHALEIN. Walter B. Shelley, Otto L. A. Schlappner and Harold B. Heiss . 118

THE GLANDS OF MONTGOMERY. William Montagna and Jeung Soon Yun . 126

THE ACTIVITY OF METABOLIC ENZYMES OF HUMAN EPIDERMIS IN RELATION TO AGE. S. Yamasawa, D. Cerimele and F. Serri 134

ADENOSINE 3′ : 5′-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE IN SKIN. I. MEASUREMENT AND PROPERTIES. P. D. Mier and E. Urselmann . . 141

ADENOSINE 3′ : 5′-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE IN SKIN. II. LEVELS IN ATOPIC DERMATITIS. S. W. J. Holla, E. P. M. J. Hollmann, P. D. Mier, W. J. B. M. v.D. Staak, E. Urselmann and J. A. Warndorff . . 147

THE CUTANEOUS REACTIONS PRODUCED BY *o*-CHLOROBENZYLIDENE MALONONITRILE AND ω-CHLOROACETOPHENONE WHEN APPLIED DIRECTLY TO THE SKIN OF HUMAN SUBJECTS. P. Holland and R. G. White 150

CEMENT DERMATITIS AND ATMOSPHERIC PARAMETERS IN KUWAIT. M. Wajdi Kanan 155

Comparative Dermatology

INNERVATION OF SWEAT GLANDS IN HORSES AND DOGS. Mary Bell and William Montagna 160

GENERALIZED CUTANEOUS ERUPTION IN A DOG, WITH HISTOLOGICAL SIMILARITY TO HUMAN MYCOSIS FUNGOIDES. D. F. Kelly, R. E. W. Halliwell and R. M. Schwartzman 164

Pharmacology and Treatment

GRENZ RAY TREATMENT OF RECURRENT HERPES SIMPLEX. A. G. Knight . 172

Case Reports

Microsporum Equinum INFECTION OF THE SCALP IN AN ADULT. K. J. O'Grady, M. P. English and R. P. Warin 175

JADASSOHN TUMOUR ARISING AT SMALLPOX VACCINATION SITE. David Porter and James Earle. 177

History of Dermatology

A HISTORY OF DERMATOLOGY IN BRISTOL AND THE WEST OF ENGLAND. Clifford D. Evans 180

No. 2.—FEBRUARY—*cont.*

Obituary

James Garlick Coburn 192

Comment

COLLAGEN, COLLAGENASE AND CLINICIANS. Gerald S. Lazarus 193

Society Proceedings

IRISH DERMATOLOGICAL SOCIETY, OCTOBER 2ND, 1970 200

Correspondence

MACULAR AMYLOIDOSIS CUTIS. J. Revuz, J. Poirier and R. Touraine . . 202
THE ACTION OF ZINC OXIDE ON DITHRANOL. Stanley Comaish and John Smith 202
ZINC AND COPPER IN PSORIASIS. Benjamin Portnoy and Mohamed M. Molokhia 205
EFFECTS OF PROPRANOLOL ON THE ACETYLCHOLINE INDUCED RESPONSE OF THE HUMAN SWEAT GLAND. H. G. W. M. Hemels, F. A. J. Thiele and F. W. Bauer 206
CANDIDA ALBICANS INFECTION AND NAPKIN RASH. P. N. Dixon, M. P. English and R. P. Warin. 207

Books

HUMAN HAIR GROWTH IN HEALTH AND DISEASE (FERRIMAN); SYMPOSIUM ON PEDIATRIC DERMATOLOGY (JACOBS, (EDIT.)) 208

Quarterly Review

THERAPEUTICS XVII. " RESERVE " DRUGS IN THE TREATMENT OF TUBERCULOSIS. Anne E. Walker, E. Frenk and A. J. Smith 210

No. 3.—MARCH

Clinical and Laboratory Investigations

PITYRIASIS LICHENOIDES: A REAPPRAISAL. R. Marks, M. Black and E. Wilson Jones 215
JUVENILE DERMATITIS HERPETIFORMIS IN JAMAICA: CLINICAL AND GASTROINTESTINAL FEATURES. Joyce Warner, S. E. H. Brooks, W. P. T. James and Sheilah Louisy 226
CHRONIC MUCOCUTANEOUS CANDIDIASIS, ENDOCRINE DEFICIENCY AND ALOPECIA AREATA. L. Stankler and P. D. Bewsher 238
DEMONSTRATION OF BASAL CELL IMMUNOFLUORESCENCE IN DRUG-INDUCED TOXIC EPIDERMAL NECROLYSIS. Kenneth M. Stein, Otto L. A. Schlappner, Charles L. Heaton and James W. Decherd 246
ROSACEA-LIKE DERMATITIS: CONTRAINDICATION OR INTOLERANCE REACTION TO STRONG STEROIDS. Gerhard Weber 253
SKIN MARKINGS IN NEGRO CHILDREN FROM THE WEST INDIES. Dorothy I. Vollum 260
THE INVESTIGATION OF PHOTOCONTACT DERMATITIS IN A DYE MANUFACTURING PROCESS. J. S. Gardiner, A. Dickson, T. M. Macleod and W. Frain-Bell . 264
SCANNING ELECTRON MICROSCOPY OF THE STRATUM CORNEUM. Rodney P. R. Dawber, Ronald Marks and J. Alan Swift 272
THE RESPONSE OF THE SWEAT GLANDS TO β-ADRENERGIC STIMULATION. J. A. Warndorff 282

A New Ultrastructural Finding in Oral Submucous Fibrosis. W. H. Binnier and R. A. Cawson 286

Pharmacology and Treatment

Out-Patient Treatment of Ichthyosis: A Double-blind Trial of Ointments. F. N. Pope, J. K. Rees, R. S. Wells and K. G. S. Lewis . . 291

Case Report

Linear Melorheostotic Scleroderma. Lawrence T. Wagers, Alex W. Young, Jr. and Stephen F. Ryan 297

Comment

The Pathogenesis of Lichen Planus. Martin M. Black 302

Toulouse 1970 306

Books

A Treatise of Skin. Vol. 1—Biophysical Properties of the Skin. (Elden (Edit.)); Cutane Paraneoplastische Syndrome (Herzberg (Edit.)) Acne Vulgaris (Frank) 308

Correspondence

"Metosyn" in Psoriasis and Eczema. B. Portnoy and I. Sarkany . . 310
The Effect of a Medicated Wash on Acne, Sebum Excretion Rate and Skin Surface Lipid Composition. W. J. Cunliffe, J. A. Cotterill and B. Williamson 311
Dapsone. J. O'D. Alexander 312

Quarterly Review

Drug Reactions XVII. Cutaneous Reactions to Barbiturates, Chloral Hydrate and its Derivatives. J. Almeyda 313

News and Notices 316

No. 4—APRIL

Clinical and Laboratory Investigations

Suppressed Cell-Mediated Immunity in Two Adults with Atopic Dermatitis. Walter C. Lobitz, Jr., Juan F. Honeyman and Norman W. Winkler 317
"Lymphomatoid" Pityriasis Lichenoides: a Variant with Histological Features Simulating a Lymphoma. A Clinical and Histopathological Study of 15 Cases with Details of Long Term Follow Up. Martin M. Black and Edward Wilson Jones 329
Ultrastructural Aspects of Dermatitis Herpetiformis (Duhring's Disease). F. Guerra Rodrigo 348
Variations in Sin Surface Lipid Composition and Sebum Excretion Rate with Different Sampling Techniques II. J. A. Cotterill, W. J. Cunliffe and B. Williamson 356
Physico-Chemical Factors Influencing Epidermal Damage by Surface Active Agents. A. B. G. Landsdown and P. Grasso 361
Localized Unilateral Hyperhidrosis—a Clinical and Laboratory Study W. J. Cunliffe, C. E. Johnson and D. M. Williamson 374

No. 4—APRIL—*cont.*

PROGRESSIVE CHANGES IN CUTICULAR PATTERN ALONG THE SHAFTS OF HUMAN HAIR AS SEEN BY SCANNING ELECTRON MICROSCOPY. Eva Bottoms, Edward Wyatt and Stanley Comaish 379

INTRACELLULAR LOCALIZATION OF TYROSINASE INHIBITOR IN AMELANOTIC AND MELANOTIC MALIGNANT MELANOMA. Toshio Hamada and Yutaka Mishima 385

ULTRASTRUCTURAL DIFFERENCES IN HUMAN MALIGNANT MELANOMATA. AN ELECTRON MICROSCOPIAL STUDY. Herbert Klug and Wolfgang Günther . 393

Pharmacology and Treatment

COMBINED CYTOSTATIC AND SURGICAL TREATMENT OF EXTENSIVE BASAL CELL EPITHELIOMATA. Gerhard Weber and Wilhelm Meinert 408

Case Reports

EXTRACTION OF PEMPHIGUS ANTIBODIES FROM A LYMPHOID NEOPLASM AND ITS POSSIBLE RELATIONSHIP TO PEMPHIGUS VULGARIS. N. K. Saikia . . 411

COIFFURE KELOIDS. Margaret A. H. Bayles 415

Comment

PRECANCER AND THE SKIN. P. C. H. Newbold 417

Obituaries

REGINALD THOMAS BRAIN 435

ARCHIBALD GORDON SMITH 436

Correspondence

THE PREVALENCE OF DERMATITIS HERPETIFORMIS. D. Burrows 437

THE PREVALENCE OF DERMATITIS HERPETIFORMIS. F. F. Hellier 438

FIXED DRUG ERUPTION DUE TO PHENAZONE IN A HYPNOTIC. Julian Verbov . 438

ZINC AND COPPER IN PSORIASIS. M. W. Greaves 439

TURKEY DIET REGIME IN THE TREATMENT OF PSORIASIS. Agnese Kelly . 440

TREATMENT OF HYPERHIDROSIS WITH TOPICAL PROPANTHELINE—A NEW TECHNIQUE. R. H. Seville and J. C. Frankland 441

Books

SJÖGREN'S SYNDROME (SHEARN); IATROGENIC DISEASES (D'ARCY AND GRIFFIN); COLOURS FOR COSMETICS. 3RD REPORT OF THE FARBSTOFF-KOMISSION OF THE DEUTSCHE FORSCHUNGSGEMEINSCHAFT; EPIDERMOLYSIS BULLOSA (GEDDE-DAHL); THE SIGNIFICANCE OF PHYSICAL SIGNS IN MEDICINE (MILLS) 442

Quarterly Review

CONTACT DERMATITIS XVIII. ANCILLARY AIDS IN ELUCIDATION OF CAUSES OF CONTACT DERMATITIS. D. S. Wilkinson 445

News and Notices 447

No. 5.—MAY

Clinical and Laboratory Investigations

TETRACYCLINE-RESISTANT β-HAEMOLYTIC STREPTOCOCCI IN DERMATOLOGICAL PATIENTS. John-Erik Brorson, Gösta Roupe and Staffan Seeberg . . 449
THE ORAL CONTRACEPTIVE AND VARIEGATE PORPHYRIA. A. W. McKenzie and U. Acharya 453
ALIMENTARY *Candida Albicans* AND NAPKIN RASHES. Peter N. Dixon, Robert P. Warin and Mary P. English 458
A MICRO METHOD FOR MEASURING THE CARBON DIOXIDE RELEASE BY SMALL SKIN AREAS. F. A. J. Thiele and L. H. J. Van Kempen. 463
THE SELECTIVE DEPIGMENTING ACTION OF 8-HYDROXYQUINOLINE ON HAIR GROWTH IN THE MOUSE. C. E. Searle 472
THE GROWTH OF ADULT HUMAN SKIN *In Vitro.* M. Levine 481
INCREASED *In Vitro* RADIO-SENSITIVITY OF MALIGNANT MELANOMA INDUCED BY THE *In Vitro* ADMINISTRATION OF CHLORPROMAZINE. Michael Cooper and Yutaka Mishima 491
CORRELATION BETWEEN VARIOUS PROTEINS OF BOVINE SNOUT EPIDERMIS. Christopher Carruthers and Malaya Bhattpcharaya 495
A COMPARISON OF *In Vitro* AND *In Vivo* METHODS OF IDENTIFYING HUMAN EPIDERMAL CELLS IN DNA SYNTHESIS. Elizabeth Hell and Howard Maibach 506
THE QUANTITATION OF KERATINIZATION IN HUMAN FOETAL TISSUES. A. Jarrett and W. Van Wyk 508
WHITE SCALP HAIRS TURNING BLACK—AN UNUSUAL REVERSAL OF THE AGEING PROCESS. Stanley Comaish 513
CONGENITAL DIFFUSE MOTTLING OF THE SKIN. D. V. Stevanović . . . 515
DAPSONE INDUCED PERIPHERAL NEUROPATHY. Edward H. Wyatt and J. Clarke Stevens 521

Pharmacology and Treatment

RETINOIC ACID IN THE TREATMENT OF PSORIASIS. Angus Macdonald and Lionel Fry 524
NAEVUS COMEDONICUS—TREATMENT WITH RETINOIC ACID. James W. Decherd, Otto Mills and James J. Leyden 528
COSMETIC CAMOUFLAGE IN DERMATOLOGY. T. W. Stewart and Doreen Savage 530

History of Dermatology

SHAKESPEARE ON THE SKIN. J. A. Cotterill 533

Comment

THE ROLE OF CHALONES IN EPIDERMAL HOMEOSTASIS. R. Marks . . . 543

Books

YEAR BOOK OF DERMATOLOGY (MALKINSON AND PEARSON (EDIT.)); DERMATOLOGY IN GENERAL MEDICINE (FITZPATRICK, ARNDT, CLARK, EISEN, VAN SCOTT AND VAUGHAN (EDIT.)) 549

Quarterly Review

THERAPEUTICS XVIII. DIURETIC THERAPY. Anne E. Walker and A. J. Smith 551

News and Notices 554

No. 6.—JUNE

Clinical and Laboratory Investigations

PATHOLOGY OF THE FINGER NAIL IN PSORIASIS. A CLINICOPATHOLOGICAL STUDY. Klaus Lewin, Sharon DeWit and Richard A. Ferrington . . 555
FURTHER EXPERIENCES OF TOXIC EPIDERMAL NECROLYSIS INCRIMINATING ALLOPURINOL, PYRAZOLONE AND DERIVATIVES. J. D. Stratigos, St. K. Bartsokas and J. Capetanakis 564
ALLERGIC CONTACT DERMATITIS FROM PYRETHRUM (*Chrysanthemum* spp.) THE ROLES OF PYRETHROSIN, A SESQUITERPENE LACTONE, AND OF PYRETHRIN II. John C. Mitchell, Gilles Dupuis and H. G. Neil Towers 568
EMOTIONS AND THE SKIN: THE CONDITIONING OF SCRATCH RESPONSES IN CASES OF ATOPIC DERMATITIS. J. M. Jordon and F. A. Whitlock 574
CHALONE CONTROL OF MITOTIC ACTIVITY IN ECCRINE SWEAT GLANDS. W. S. Bullough and Johanna U. R. Deol 586
THE EFFECT OF OCCLUSIVE AND SEMIPERMEABLE DRESSINGS ON THE MITOTIC ACTIVITY OF NORMAL AND WOUNDED HUMAN EPIDERMIS. Louis B. Fisher and Howard I. Maibach 593
NUCLEAR AND NUCLEOLAR ULTRASTRUCTURE DURING THE LATE STAGES OF NORMAL HUMAN KERATINOCYTE MATURATION. J. Karásek, K. Smetana, A. Hrdlička, I. Dubinin, O. Horňák and W. Ohelert 601
FLUORIMETRIC ASSAY OF CARBOXYLIC ESTER HYDROLASES IN GUINEA-PIG SKIN AND THE EFFECTS OF PROPRANOLOL. D. W. K. Cotton and José J. M. A. van den Hurk 608
LIPID SYNTHESIS (^{14}C-ACETATE INCORPORATION) IN THE ISOLATED HUMAN SEBACEOUS GLAND, THE APPENDAGE-FREED EPIDERMIS, THE SEBOCYST (STEATOCYSTOMA) AND THE WEN (KERATINOUS CYST OF SKIN). Roy Summerly and Sheila Woodbury 614

Pharmacology and Treatment

IS SULPHUR HELPFUL OR HARMFUL IN ACNE VULGARIS? Otto H. Mills, Jr. and Albert M. Kligman 620
TOPICAL TREATMENT WITH BAY B 5097, A NEW BROAD SPECTRUM ANTIMYCOTIC AGENT. Torsten Fredriksson 628
HYPERBARIC OXYGEN THERAPY IN DERMATOLOGY. P.-O. Barr, Wera Enfors and Gunner Eriksson 631

Case Reports

GRANULOMA ANNULARE IN IDENTICAL TWINS. S. K. Goolamali and C. J. Stevenson 636
RECURRENT ECZEMA HERPETICUM ASSOCIATED WITH ICHTHYOSIS VULGARIS. Julian Verbov, D. D. Munro and Ann Miller 638

Review

A REVIEW OF THE RECENT LITERATURE ON DISEASES OF THE VULVA—PART I. GENERAL ASPECTS. C. M. Ridley 641

Books

PROGRESS IN ALLERGY. VOL. 15 (KALLOS, WAKSMAN AND DE WECK (EDIT.)); RESISTANCE TO INFECTIOUS DISEASE (DUNLOP AND MOON (EDIT.)) . . 648

No. 6—JUNE—*cont.*

Correspondence

AN ASSESSMENT OF DAPSONE TOXICITY IN THE GUINEA-PIG. M. H. Williams and W. G. Bradley 649

Quarterly Review

DRUG REACTIONS XVIII. CUTANEOUS REACTIONS TO ANTITUBERCULOUS DRUGS. Aslhey Levantine and John Almeyda 651

Index Volume 86 and Supplement No. 8 656

SUPPLEMENT No. 8

Foreword 1

Acknowledgments 2

THE BACTERIOLOGY OF ACNE. J. A. Savin 3

THE RELEVANCE OF SKIN SURFACE LIPIDS TO ACNE VULGARIS. W. J. Cunliffe, J. A. Cotterill, B. Williamson and R. A. Forster 10

THE MICROBIOLOGY OF THE CUTANEOUS DIPHTHEROIDS. D. A. Somerville. . 16

COLONIZATION WITH STAPHYLOCOCCI IN DERMATOLOGY WARDS WITH DIFFERENT DESIGNS AND PRACTICES. B. Nyström and L. Molin 21

AN ANIMAL MODEL FOR TOXIC EPIDERMAL NECROLYSIS. H. M. Dick and J. E. Baird 28

STUDIES ON STAPHYLOCOCCAL TOXINS IN RELATION TO TOXIC EPIDERMAL NECROLYSIS (THE SCALDED SKIN SYNDROME). J. P. Arbuthnott, J. Kent, A. Lyell and C. G. Gemmell. 35

ACTION OF STAPHYLOCOCCAL EPIDERMOLYTIC TOXIN ON MOUSE SKIN IN ORGAN CULTURE. H. M. McCallum 40

THE PATHOGENIC ROLE OF COAGULASE NEGATIVE STAPHYLOCOCCI. R. J. Holt . 42

IMPETIGO CONTAGIOSA IN THE UNITED KINGDOM. B. L. Connor 48

STREPTOCOCCAL PYODERMA AND ACUTE NEPHRITIS IN TRINIDAD. D. C. J. Bassett 55

IMMUNOLOGY OF STREPTOCOCCAL INFECTION. G. M. Levene 62

DISTRIBUTION OF DERMATOPHYTES AND CANDIDA SPORES IN THE ENVIRONMENT. G. Midgley and Y. M. Clayton 69

THE EPIDEMIOLOGY OF ANIMAL RINGWORM IN MAN. M. P. English 78

MUCOCUTANEOUS CANDIDIASIS. J. Higgs and R. S. Wells 88

Clinical and Laboratory Investigations

Br. J. Derm. (1972) **86**, 1.

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A SURVEY OF ICHTHYOSIS VULGARIS IN ISRAEL

L. ZIPRKOWSKI AND A. FEINSTEIN

SUMMARY.—Three hundred and sixty-six patients with ichthyosis vulgaris were examined, including 229 with X-linked recessive ichthyosis (X.L.I.) and 137 with autosomal dominant ichthyosis (A.D.I.), among the Jewish population of Israel. The minimal incidence of the disease in this population is about 1 : 6500. The disease affected the various ethnic groups differently; among Iraqi Jews there were cases only of X.L.I. compared with only A.D.I. among Indian Jews. The disease appeared in both forms in 95% of cases before 5 years of age, and half of the cases showed improvement in the course of time; 80%–95% of the patients noted improvement in the summer months. A family history of " atopy " was found only in about a third of cases of A.D.I. and in none of those with X.L.I.

The most pronounced clinical features of the disease were found over the anterior aspect of the legs, and equally in both forms. Follicular keratosis of the arms and hyperkeratosis with prominent creases of the palms and soles were found only in A.D.I.

The histological findings were diagnostic, especially in those areas where the scaling was obvious, and thus it is recommended to biopsy the anterior shin region. The histology in X.L.I. showed compact laminated hyperkeratosis, with thickening of the granular layer; whereas, in A.D.I., the hyperkeratosis was usually of basket-weave type, with a thin or absent granular layer. The sweat glands were normal in structure and number in both forms, while the sebaceous glands were absent only in cases of A.D.I.

ICHTHYOSIS was known in ancient times, and the name is derived from the Greek word: *ichthys*, meaning fish. The disease is not rare and in fact forms one of the largest groups of the genodermatoses.

Approximately 95% of all types of ichthyosis can be included under the title ichthyosis vulgaris (Wells and Kerr, 1966a). Since the 1930's ichythosis vulgaris has been known to be transmitted either by autosomal dominant or by X-linked recessive inheritance (Orel, 1929; Bruhns, 1931; Cockayne, 1933). However, only in the last 4 years have clinical and histological differences between the 2 inherited forms been described (Wells and Kerr, 1966a). The purpose of this survey was to assess the incidence of ichthyosis vulgaris in Israel, and to describe and compare the clinical and histopathological differences between the autosomal dominant and the X-linked recessive forms.

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MATERIAL AND METHODS

Between 1965 and 1970, 366 patients with ichthyosis vulgaris were examined. All were of Jewish origin and came from 139 families. The patients were collected from 2 sources: (1) records of all the hospitals in Israel; (2) referrals from all dermatologists in Israel, following a request circular.

The 366 patients consisted of 229 with X-linked recessive ichthyosis (X.L.I.) and 137 with autosomal dominant ichthyosis (A.D.I.). They were examined clinically and requested to provide details regarding the progress of the disease. In 103 cases a biopsy was performed, 71 in cases of X.L.I. and 32 in A.D.I. The biopsies were taken from the arm, trunk or shin, and the preparations were stained with HE.

RESULTS

Altogether 366 patients were examined, including 229 males with X.L.I., and 137 patients with A.D.I., consisting of 67 male and 70 female cases. The age distribution is shown in Table I

TABLE I —*Age Distribution of Patients with Ichthyosis Vulgaris*

Age	No. of patients with X.L.I.	No. of patients with A.D.I.
0–9	73	38
10–19	101	38
20–29	23	35
30–39	16	6
40–49	6	9
50–59	10	9
60–69	—	2
Total	229	137

TABLE II —*Ethnic Origin of Patients*

Country of origin	No. of patients with X.L.I.	No. of patients with A.D.I.
Germany	17	—
Russia	8	6
Poland	17	10
Hungary	6	4
Czechoslovakia	—	6
Roumania	16	14
Yugoslavia	3	—
Greece	9	14
Turkey	3	—
Syria	17	2
Iraq	56	—
Egypt	6	—
Morocco	29	18
Libya	6	6
Yemen	16	6
Iran	20	2
India	—	49
Total	229	137

Table II shows the distribution of the cases according to their country of origin.

Table III shows details regarding age of onset, progression of the disease, improvement in the summer months and the incidence of atopy in the family (hay-fever, asthma, etc.).

Clinical findings

Generally the disease was more prominent in those patients with X L.I., presenting with widespread scales, which themselves were larger and darker. The scales in these cases covered most of the trunk and limbs. In A.D.I. mainly the legs were involved, and the upper extremities to a lesser degree. The trunk was rarely affected, and then only mildly.

Face.—X.L.I. commonly involved the forehead adjacent to the hairline, the ears, the pre-auricular and post-auricular regions and the angle of the mandible. In A.D.I., however, the face was usually uninvolved and only occasionally were fine powdery scales noted.

TABLE III —*Historical Data*

	X.L.I. (%)	A.D.I. (%)
1. Age of onset of the disease		
At birth	60	64
Up to 6 months	15	15
6–12 months	10	—
1–5 years	10	15
More than 5 years	5	6
2. Course of the disease		
Without change	45	50
Improvement with time	55	50
3. Improvement in summer		
Marked improvement	56	56
Slight improvement	39	24
Without change	5	20
4. " Atopy " in the family	—	30

TABLE IV.—*Region and Degree of Involvement*

	X.L.I.	A.D.I.
Face	+	—
Neck	+ +	—
Upper part of the body	+ +	—
Lower part of the body	+ +	+
Upper limbs	+ +	+
Lower limbs	+ + +	+ + +
Antecubital and popliteal fossae	±	—
Hands and feet	—	+ +

Neck.—The neck was commonly involved in X.L.I., with pigmented scales, but usually spared in A.D.I.

Trunk.—The trunk was involved in X.L.I., sometimes circumferentially, and especially over the loins and sacrum. The vertebral column was usually free. In A.D.I. the loins were affected as well as the lower portions of the trunk, while the upper trunk was free.

Limbs.—The limbs were widely affected, especially the extensor surfaces in X.L.I. and A.D.I. However, in X.L.I. other parts of the body were more affected than in A.D.I. The anterior part of the leg was equally affected in X.L.I. and A.D.I. The antecubital and popliteal fossae were always spared in A.D.I., whereas in X.L.I. they were sometimes mildly affected.

Follicular keratosis was detected on the upper arms of some cases of A.D.I., but never in X.L.I.

Palms and Soles.—A further characteristic of A.D.I. was the finding of hyperkeratosis of the palms and soles, with prominent creases and fissuring. In all cases of X.L.I. the palms and soles were unaffected.

The degree of involvement related to body distribution is shown in Table IV.

Scales.—The size, shape and colour of the scales were related to the age of the patient, his ethnic group and the part of the body involved. In children the scales were smaller, and the colour was a dirty grey, brown or black. In dark-skinned patients the colour was darker. Usually the colour of the scales was lighter in adults (perhaps due to decreasing severity of the disease with passing years).

In X.L.I. the scales were 1–3 cm in size. They were large, thick and, at their centre, with free edging. The colour was usually dark-grey, brown or black. Their shape was polygonal, with 4–5 sides, and arranged in a mosaic fashion. On the trunk they were often organized linearly in a cephalo-caudal direction, and were finer and thinner.

TABLE V.—*Comparison of Scales*

	X.L.I.	A.D.I.
Size:	1 cm–3 cm	1 mm–1 cm
Colour:	Grey-brown-black	Grey-brown
Shape:	Polygonal (4–5 edges)	" Powdery " or polygonal (4–5 edges)

In A.D.I. the scales were usually smaller (up to 1 cm), finer and lighter in colour. The scaling was a dirty-grey to brown colour, but never black. They were also polygonal, with 4–5 sides, but less well organized and, in most cases, isolated with an indistinct border. In other areas they were smaller and lighter and had a powdery look. Overlying the shins the scales were thicker, darker and arranged in a mosaic fashion, indistinguishable from X.L.I. A comparison of the scales in the 2 forms is presented in Table V.

Histology

There were histopathological differences between the biopsies from X.L.I. and A.D.I. cases. These were especially marked in those biopsies taken from areas with thick, obvious scaling (in X.L.I. from the shin, trunk or arm; in A.D.I. from the shin). Biopsies from lightly affected areas (e.g. the trunk or arms in A.D.I.) did not always show unequivocal changes and often resembled normal skin. Most of the histological differences were found in the stratum corneum and granular layer. The stratum corneum was always hyperkeratotic. In X.L.I. the hyperkeratosis was always compact and laminated, while in A.D.I. it was basket-woven, although in some areas compacted and laminated. The granular layer was usually absent or 1 layer thick in A.D.I.; but in X.L.I. 3–4 and often 5–6 layers were present. The Malpighian layer was usually intact, but in some cases of X.L.I. there was marked papillomatosis.

A further interesting finding was observed with regard to the sebaceous glands. In all the cases of X.L.I. the sebaceous glands were present, while in A.D.I. sebaceous glands were found in only 2 of 32 histological preparations, and those present were small and atrophic. In preparations from both forms of ichthyosis, sweat glands and hair follicles were present. In most cases there was a slight lympho-histiocytic infiltration around the blood vessels. A summary of the histological findings appears in Table VI (Fig. 1–3).

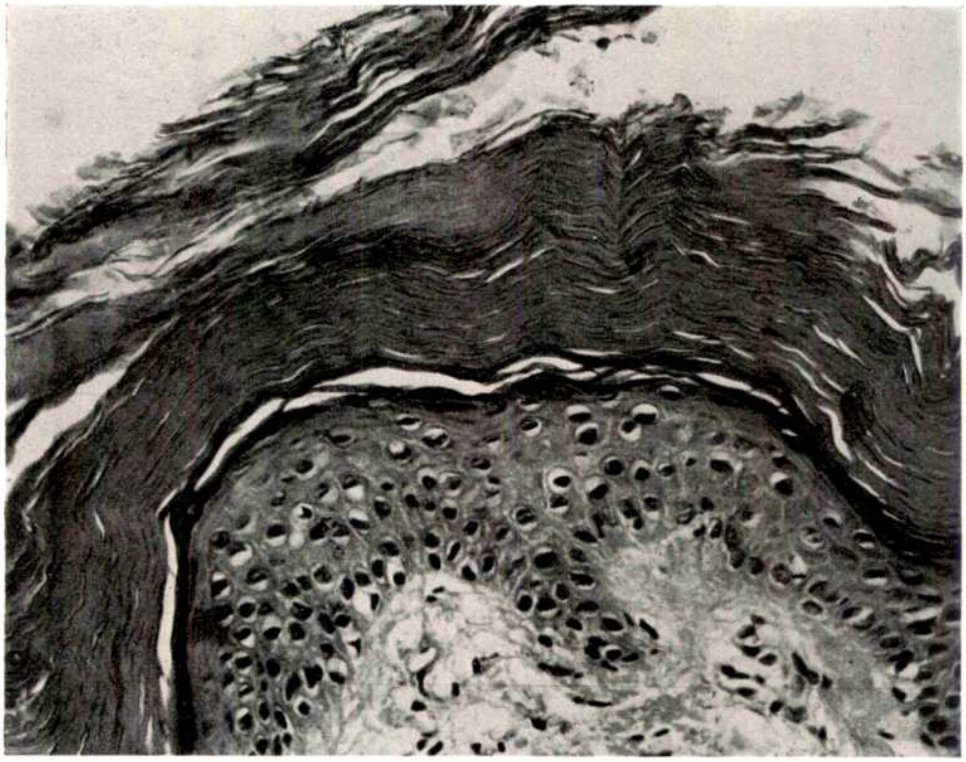

FIG. 1.—Compact, laminated hyperkeratosis and a granular zone 3 cells thick from a patient with X-linked ichthyosis (× 360).

TABLE VI.—*Histological Findings*

	X.L.I.	A.D.I.
Horny layer:	Compact laminated hyperkeratosis	Basket-weave hyperkeratosis (sometimes compact laminated hyperkeratosis)
Granular layer:	3–4 cell thickness	0–1 cell thickness
Malpighian layer:	Normal (sometimes marked papillomatosis)	Normal
Inflammatory infiltration:	Poor infiltration around blood vessels	Poor infiltration around blood vessels
Sweat glands:	Present	Present
Hair follicles:	Present	Present
Sebaceous glands:	Present	Absent

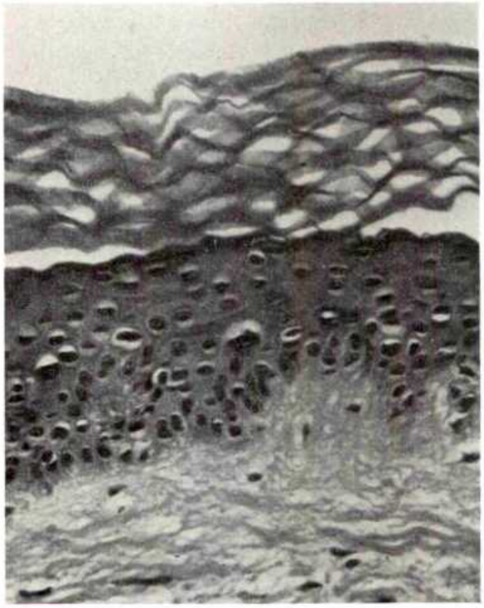

FIG. 2.—Basket-weave hyperkeratosis, with absence of granular layer, from a patient with autosomal dominant ichthyosis (× 360).

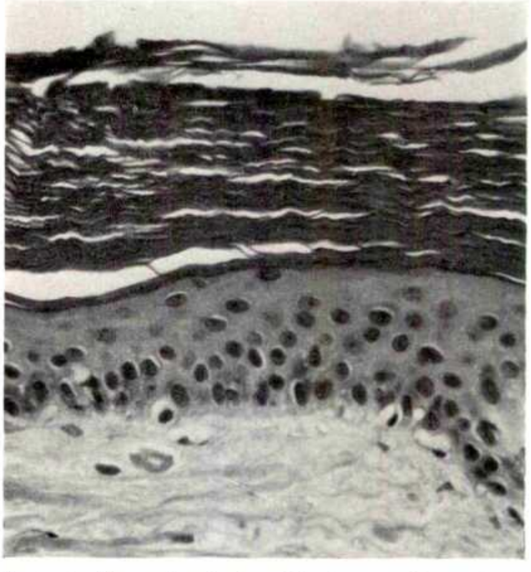

FIG. 3.—Compact, laminated hyperkeratosis, with absence of granular layer, from a patient with autosomal dominant ichthyosis (× 360).

DISCUSSION

In the 5 years from 1965–1970, 366 cases of ichthyosis vulgaris in Jews were examined in Israel. Considering that the average Jewish population at that time was 2,380,000, the minimal incidence of the disease can be calculated at 1 : 6,500. Wells and Kerr (1966a) in England found an incidence of 1 : 3,665.

Most of the cases fell into the younger age group, probably due to increased vigilance amongst this group, which prompted them to consult a physician. In the younger patients the disease caused a great deal of suffering, due primarily to environmental factors, whereas the adults tended to be apathetic towards their disease. The fact that the disease tends to improve with age also reduced the number of cases in older age groups seeking consultation. The disease included cases of West and East European as well as Near and Far Eastern origins (Table II). However, there were some obvious differences in the epidemiological features relating to the mode of inheritance. Although about 25% of the cases of X.L.I. came from Iraq (56 of 229), no Iraqis Jews were found among the A.D.I. This was also true for cases of German, Yugoslavian and Egyptian extraction. Indian Jews were solely of the dominant type, forming more than 35% of the cases (49 of 137). This difference in the incidence of the 2 forms of the disease according to country of origin is well known in the literature. Cockayne (1933) found an increased incidence of the dominant variety in Scandinavia, England and Germany, while the X-linked form was more common in the Latin countries. Ito (1950) found that all the cases of ichthyosis vulgaris in Japan belonged to the dominant group, similar to our findings regarding Indian Jews.

Among our cases we did not find any significant difference in the age of onset in the 2 inherited forms (Table III). Previously it was thought that, in cases of X.L.I., the onset was at an earlier age than with A.D.I. (Wells and Kerr, 1966a; Kuokkanen, 1969). The great majority of our cases, regardless of type (94% or 95%), appeared by the age of 5 years, and only a small minority (5% or 6%) appeared after this age. Only in isolated cases in the literature has the onset occurred at an older age. Heller (1921) described a family where the disease appeared for the first time after 30 years. Half the cases described improved in their condition with the passage of time; 95% of patients with X.L.I. and 80% with A.D.I. mentioned slight to marked improvement in the summer months. A family history of atopy (hay fever, asthma or atopic dermatitis) was mentioned only in those cases with the autosomal dominant form of ichthyosis.

The clinical findings resembled those in previous surveys of ichthyosis (Wells, 1966; Wells and Kerr, 1966a; Kuokkanen, 1969). In X.L.I. the disease was more severe and widespread, and the scales were darker and larger (Table IV, V). Of special note are the follicular keratoses mainly on the arms and the hyperkeratoses with increased skin creases and fissures on the palms and soles found in many cases of A.D.I. but not in X.L.I. The palms and soles in children and young patients with A.D.I. so resembled elderly people that the diagnosis of A.D.I. could be made on this finding alone. Although other parts of the body were more severely affected in X.L.I., the shin region of the legs was maximally and equally affected in X.L.I. and A.D.I. In this region it was impossible to distinguish the 2 forms.

Histology was taken from different areas of the body (back, arms and shins). The findings were characteristic in those places where the scaling was thick and plentiful. Where the scaling was scanty the findings were not so typical, and often resembled normal skin. We therefore recommended taking a diagnostic biopsy from the anterior surface of the shin where the scaling is the thickest.

The histological findings in both forms correspond to most of the features described in previous works (Wells and Kerr, 1966b; Kuokkanen, 1969) and have been published in a separate paper (Feinstein *et al.*, 1970). In X.L.I. there was compact laminated hyperkeratosis with hyperplasia of the granular layer, whereas in A.D.I. the hyperkeratosis was of basket-weave type, with occasional foci of compact laminated hyperkeratosis, and the granular layer thin or absent. It is noteworthy that some cases of X.L.I. showed marked papillomatosis.

Previous works on the histology of ichthyosis vulgaris (Blum, 1936; Degos, 1953; Gans and Steigleder, 1955; Lever, 1961; Percival *et al.*, 1962; Montgomery, 1967) stress that the number of sweat and sebaceous glands is reduced in both forms. The most recent articles by Wells (1966) and Wells and Kerr (1966b) also confirm the reduction in the number of sweat glands in ichthyosis vulgaris. Our findings do not support this observation. In our survey there was no reduction in the number of sweat glands in any of the histological preparations from either form of the disease. Wells and Kerr (1966b) described a decrease in the number of sebaceous glands in some cases of A.D.I. However, our findings showed a marked and unequivocal difference between the 2 forms. In all cases of X.L.I. sebaceous glands were found, compared with none in cases of A.D.I. (excluding the 2 cases where a scanty number of atrophic glands were demonstrated). In all these cases several serial sections were examined.

In view of these findings we think that the sweat glands which appeared in normal numbers were not responsible for the clinical findings. Probably the absence of sebaceous glands in A.D.I. is responsible for the dry skin in these cases. In X.L.I., where the sebaceous glands are present, it would be most important to investigate their functional capacity to secrete sebum.

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DISSEMINATED ELASTOSIS PERFORANS SERPIGINOSA IN FOUR MONGOLOIDS

RECOGNITION OF RESIDUAL CHANGES

JAMES E. RASMUSSEN

SUMMARY.—Disseminated elastosis perforans is described in 4 mongoloids. The natural evolution of the disorder in 3 of these patients has produced linear and retiform scars. Recognition of this sequela will facilitate diagnosis when typical grouped or annular hyperkeratotic papules are no longer present.

Since Lutz (1953) described elastosis perforans serpiginosa (EPS) in 1953, more than 100 patients have been reported with this disorder. Haber (1959) and Mehregan (1968) characterized in their reviews the clinical features of EPS as grouped, annular or arciform hyperkeratotic papules through which, Mehregan has proposed, abnormal elastic fibres are extruded via " transepidermal elimination ". The distribution of these lesions has also been considered a reliable characteristic of EPS. Seventy-six of the 101 patients reviewed by Mehregan (1968) had papules confined to a single anatomical site, usually the nape of the neck or upper extremities. Both Haber (1959) and Mehregan (1968) noted an association of EPS with mongolism, and disorders of connective tissue such as Ehlers-Danlos syndrome, osteogenesis imperfecta and Marfan's syndrome. Mehregan noted that, in the 4 cases associated with pseudoxanthoma elasticum, EPS occurred in a more generalized fashion, involving the chest, back and extremities. Only 2 previous English language publications, however, has fully described a mongoloid patient with wide-spread lesions of EPS (Ritchie, 1960; Stritzler, 1967).

The purpose of this report is to describe 4 mongoloids with a disseminated form of EPS and to chronicle its natural evolution.

CASE REPORTS

The patients were seen at the Fairview Hospital and Training Center for the mentally retarded, Salem, Oregon, during the period 1968–1971. All 4 are moderately retarded mongoloids. Three are boys. Pertinent details of the patients are given in Table I.

Patient I.—In 1968 he had grouped, annular and isolated, brightly erythematous, hyperkeratotic papules bilaterally, but randomly distributed on the posterior portion of the neck and on the upper and lower extremities (Fig. 1). By 1971 the eruption had changed remarkably without therapy. The grouped and annular lesions were no longer present. The thighs and upper extremities were etched with linear and retiform hypopigmented scars, several of which contained a hyperkeratotic papule at one end (Fig. 2, 3). Isolated papules were numerous. A biopsy specimen of a lesion at one end of a scar

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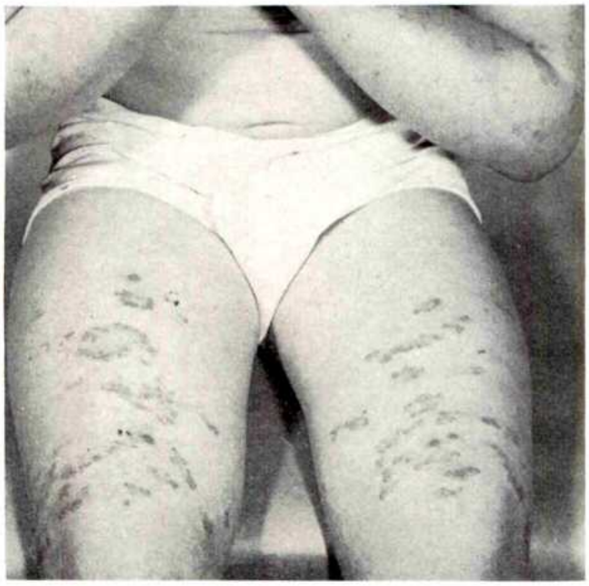

FIG. 1.—Patient I in 1968. Grouped and annular collections of papules are shown on upper and lower extremities.

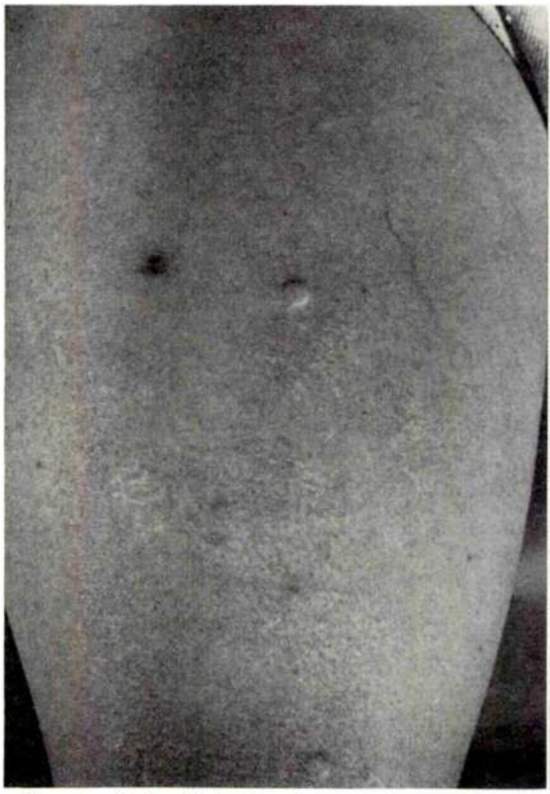

FIG. 2.—Right thigh of Patient I in 1971. Lesions shown in Fig. 1 have resolved, leaving linear and retiform scars. Also note hypertrophic scar on upper thigh at site of previous biopsy.

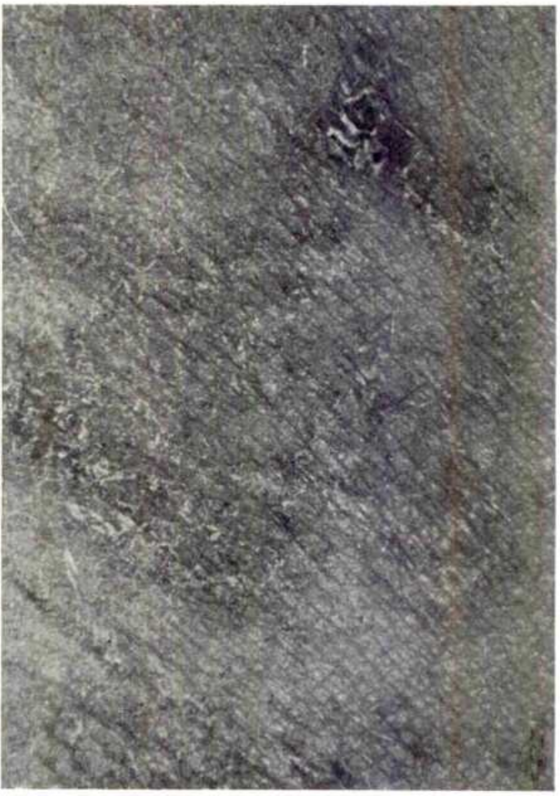

FIG. 3.—Right forearm of Patient I in 1971, demonstrating retiform scar with hyperkeratotic papule at upper margin.

TABLE I

	Patient I	Patient II	Patient III	Patient IV
Age	13	16	12	14
Sex	Male	Male	Male	Female
Probable duration	5 years	1–2 years	1–2 years	3–4 years
Initial manifestations	Grouped, annular hyperkeratotic erythematous papules on neck, upper and lower extremities.	Same	Same	Same
Natural evolution of disease	Spontaneous resolution, with retiform scarring often containing a single papule at one end. Numerous isolated papules also present. Hypertrophic biopsy scar.	No observable change in 6 months. Hypertrophic biopsy scar.	Original lesions predominate, but many show progression to short, linear scarring.	Numerous linear scars containing a single hyperkeratotic papule at one end. Many papules not associated with scars.

showed the transepidermal channel containing basophilic debris and eosinophilic fibres characteristic of EPS.

Patients II and III.—These boys were seen in late 1970 and closely resembled the original description of Patient I in relation to degree of mental impairment as well as configuration, distribution and extent of involvement with the inflammatory keratotic papules. Biopsy specimens were diagnostic of EPS. Patient III also shows linear scars punctuated by a typical papule at one end.

Patient IV.—She had her grouped papular lesions for at least 3 years. In January 1971 typical annular lesions were no longer present. Instead she had numerous erythematous hyperkeratotic papules situated at one end of linear or retiform scars on the sides of the neck, the extensor portion of the upper extremities and the anterior aspects of the lower limbs (Fig. 4, 5). The histopathologic diagnosis of the solitary papules was EPS (Fig. 6).

DISCUSSION

These 4 patients represent 1% of the population with Down's Syndrome at the Fairview Hospital and Training Center.

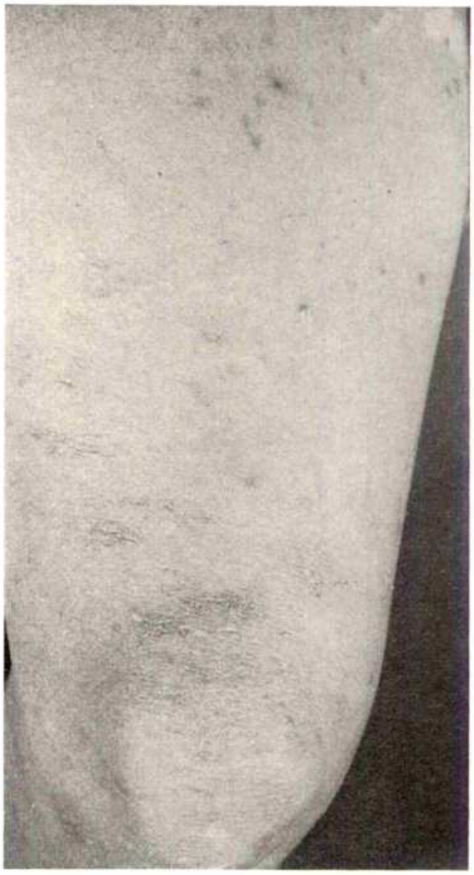

FIG. 4.—Left thigh of Patient IV in 1971. Inflammatory papules have spontaneously regressed producing numerous linear scars.

Whether this is any index of the true incidence of EPS in the general mongoloid population must await further study of larger numbers of these mentally retarded patients.

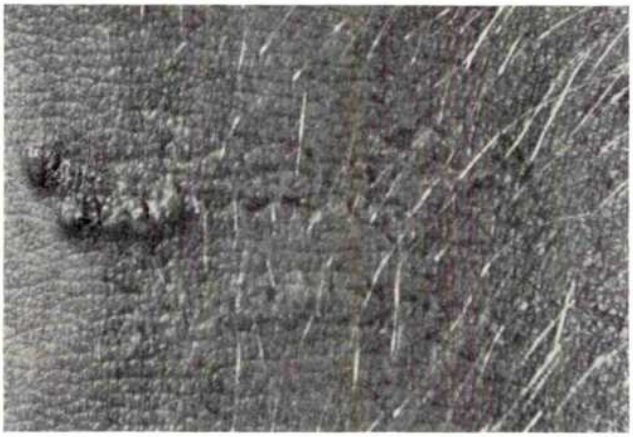

FIG. 5.—Right forearm of Patient IV in 1970. Natural evolution of the hyperkeratotic papules on the left have produced the scarred channels shown in the central and right portions of this photograph.

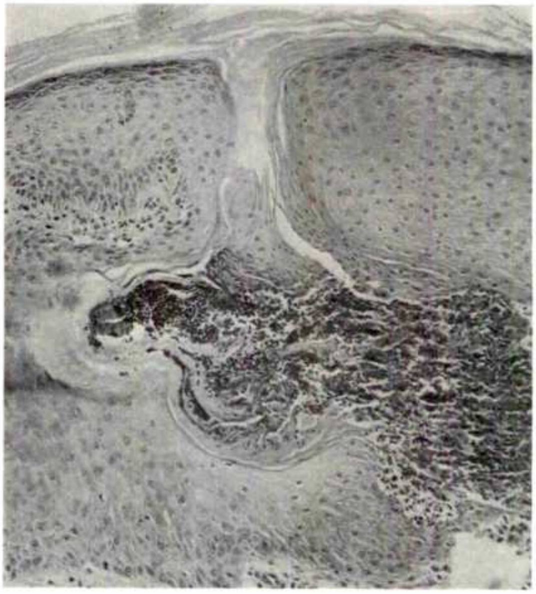

FIG. 6.—Photomicrograph of papule from Patient IV demonstrating transepidermal channel containing basophilic debris. (H. and E. × 100).

A consideration of these patients suggests that EPS need not be characterized as a process restricted to a solitary anatomic location. In addition, the natural progression of EPS can produce linear and retiform scars which contain a typical hyperkeratotic erythematous papule. This is a finding which can assist in clinical diagnosis when the characteristic annular papules are no longer present, and when a high degree of suspicion must exist before a physician is motivated to order elastic tissue stain and multiple sectioning of specimens which frequently are necessary to demonstrate the diagnostic pathology. The previous description and photographs should provide this motivation.

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EPIDERMAL "TURNOVER TIME"—A RE-EXAMINATION

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SUMMARY.—The literature on the turnover or renewal time of normal and psoriatic epidermis is critically reviewed. It is concluded that the time taken by a cell to travel to the surface, once it has been formed, is probably 40–56 days in normal skin, and 6–8 days in psoriatic skin.

It is commonly stated that the normal human epidermis requires approximately 27–28 days to renew itself or "turn over", while the psoriatic lesion requires only 3–4 days (e.g. Van Scott and Farber, 1971). This time in the normal epidermis is said to be evenly divided between the time needed for a cell to travel from the germinative (basal) layer to the stratum corneum (14 days) and the time needed for passage through the stratum corneum (14 days).

Recent data (Weinstein and Frost, 1971), however, indicate that the average normal epidermal germinative cell with a DNA synthesis time of 16 h takes approximately 457 h or 19 days to reproduce. I think it is evident that, if it takes 19 days for the germinative layer to reproduce once, it cannot possibly renew the entire viable malpighian compartment in only 14 days.

The purpose of this paper is to present arguments from the literature which indicate that the time needed for replacement of the malpighian compartment of the epidermis is much longer than 14 days, and to review the historical development of the commonly accepted values.

Definitions and Initial Approach

"Turnover time" is synonymous with "regeneration time" or "renewal time" or "replacement time" and refers to the *average* time it takes for a cell population to reproduce itself (see Table I). There is a turnover time for the germinative cell layer, which refers to the average time needed for a cell (or all cells) to make a new daughter cell (or a new germinative cell layer). There is a turnover time for the viable epidermis, which refers to the average time needed for replacement of the entire epidermis from the germinative through the granular layers. This is identical to, or synonymous with, the time needed for replacement of the germinative cell layer plus the average time needed for a cell after being formed to travel from the germinative layer to the upper stratum granulosum. There is also a turnover time for the stratum corneum, which is the time needed for the passage of a cell from the bottom of the stratum corneum to the top, and is equivalent to the *average transit time* through the stratum corneum. Finally, there is the total epidermal replacement or turnover time, and this is the sum of the turnover times of the viable epidermis and the stratum corneum.

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Probably the most direct approach to answering the problem of the total time needed for epidermal replacement is to consider first the rate of formation of new cells by the germinative layer. If we then know what proportion of the total viable epidermal cells are germinative, we can calculate the time needed for replacement of the viable epidermis. Finally, we must know the time needed for a cell to pass through the stratum corneum.

What is the Germinative Cell Replacement Time?

Weinstein and Frost have painstakingly determined the average time needed for a germinative cell to divide and produce a new cell. In 1968 their original estimate was 37·5 h for psoriasis and over 163 h for normal epidermis. In 1969, with more data on normal epidermis, the figure was 308 h, while their most recent determination (1971) for normal epidermis is 457 h, or 19 days.

How Many Cell Layers are Germinative?

This is somewhat more difficult and perhaps may still not be entirely settled. Pinkus, for example, (1952) noted that 15 of 38, or 39%, of all dividing cells in normal epidermis were above the basal layer, and he reviewed extensive evidence that many dividing cells are above the basal layer (Pinkus, 1954). The most recent study of this problem is that of Penneys *et al.* (1970) who made serial sections and radioautographs on normal skin injected with tritiated thymidine 1 h prior to removal. They noted that, although 68% of all cells undergoing DNA synthesis were in the basal layer, 32% of all cells undergoing DNA synthesis were above the basal layer. It would, therefore, seem that, for every 2 cells in the basal layer which are undergoing division, there is 1 which is above the basal layer, which is also dividing. The germinative cell population can, therefore, best be considered to consist of 1½ cell layers in normal human epidermis.

TABLE I.—*Epidermal Population Kinetics*

	Normal	Psoriasis
Total epidermal renewal or turnover time (days)	52–75	8–10
Stratum corneum renewal or turnover time (days)	14	2
Malpighian cell renewal or turnover time (average transit time) (days)	26–42	4–6
Germinative layer Renewal or turnover time (days)	12–19	1·5 (37·5 h)
Transit time (minimal through the malpighian layers (days)	14	2

How Many Total Living Cell Layers are in the Normal Epidermis?

There is a surprising lack of information on this point, but I think most histologists would accept that there are at least 6 cell layers, if the stratum granulosum is included, and certainly the normal epidermis illustrated by Epstein and Maibach (1965) and by Weinstein and Van Scott (1965) contains 6 or 7 layers.

Does One Germinative Cell Layer Form Only One Layer Higher Up in the Malpighian Layer?

From the above considerations it could be concluded that, if it takes 19 days for the germinative cell population to produce 1½ more cell layers, then 3 such generations (or 57 days) would be needed to replace the 4½ cell layers lying above the germinative cell layers. However, this is true only if there is a 1 to 1 correspondence between germinative cell layers and those situated at higher levels. The data of Pinkus (1952) indicate that approximately 31% of all nuclei are basal-appearing (or germinative). If there was a 1 to 1 correspondence between the germinative layers ($1\frac{1}{2}$ cells layers or 25% of the total viable cells) and the higher layers ($4\frac{1}{2}$ cell layers or 75% of the total viable cells), then only 25% of the nuclei should be germinative. It would appear, therefore, that, as the cells move up, they become larger, and each germinative cell layer actually contributes to more than 1 layer at higher levels. If we correct for this by using the data of Pinkus ($25/31 = X/3$; $X = 2{\cdot}2$), the germinative cell layer would have to reproduce only 2·2 times to replenish the upper layers and this would reduce the time needed from our figure of 57 days to approximately 42 days for replacement of the viable epidermis above the germinative cell layer.

How Long Does it Take for Cells to Pass Through the Stratum Corneum:

Unlike the situation in the viable epidermis, where cells are released to pass upward in a random fashion (Epstein and Maibach, 1965; Weinstein and Van Scott, 1965), once they get to the stratum corneum they probably are tightly attached to one another and all travel in unison. There is little direct proof for this, except for the autoradiographs published by Weinstein (1965) and by Frost *et al.* (1966), which show a beautiful band of radioactive protein moving up through the stratum corneum after the administration of radioactive glycine. Assuming that this is correct and that the cells do move in unison through the stratum corneum, then in this compartment of the skin the cells all move at the same speed, and minimal, maximal and average transit times are all the same.

Under these circumstances, if a radioactive amino-acid which is incorporated into protein at the level of the granular layer is administered and the time of first appearance of radioactive protein at the surface is monitored, this will give the average transit time or turnover time of the stratum corneum. Glycine is such an amino-acid, and in normal epidermis it appears at the surface incorporated into protein at 14 days according to both Rothberg *et al.* (1961) and Porter and Shuster (1967). Collaborative evidence has also been provided by Baker and Kligman (1967), who followed the disappearance of fluorescence from the stratum corneum after it had been permeated by a fluorescent dye. They reported that, for the skin of the back, this process took an average of 15·3 days; and, for the forearm, 13·3 days. Fourteen days, therefore, for passage through the normal stratum corneum seems well established. The comparable figure for psoriasis is 2 days (Rothberg *et al.*, 1961; Weinstein and Van Scott, 1965), although Porter and Shuster (1968) found activity in 1 day.

In summary, if the data of Weinstein and Frost (1971) are correct, the total epidermal regeneration time should be approximately 75 days in normal epidermis (19 days for the germinative cell layer plus 42 days for the overlying viable epidermis and 14 days for the stratum corneum) and the time needed for a cell once formed to be lost at the surface would be 56 days (42 days for the stratum malpighii and stratum granulosum and 14 days for the stratum corneum). If DNA synthesis time is not 16 h but 10 h, as recently suggested by Heenan and Galand (1971), then total epidermal renewal time would be reduced to 52 days (12 days for the germinative cell layers, 26 days for the overlying viable cells, and 14 days for the stratum corneum), and the time needed for a cell once formed to be lost at the surface would be 40 days. These figures are given in Table I. These figures are far enough removed from the usually accepted 28 days for normal epidermis to require some examination of past studies and the reasons for the discrepancies.

HISTORICAL REVIEW

Before the advent of radioactive tracers the only information which could be obtained was the number of mitoses (or the mitotic index) present in the epidermis, and this literature has been reviewed (Pinkus 1952, 1954; Thuringer and Katzberg, 1959). Unfortunately, to estimate the turnover time from the mitotic index requires knowledge of the duration of mitoses, and for technical reasons this part of the mitotic or generative cycle is the most difficult to determine with accuracy (Weinstein and Frost, 1969). If, for example, one accepts a mitotic index of 1·6 per 1,000 cells as found by Pinkus (1952), and computes the regenerative time of the viable epidermis as did Rothberg *et al.* (1961) a figure of 26 days is obtained, assuming a mitotic duration of 1 h, and 52 days if it takes 2 h for a cell to complete mitosis. The respective total epidermis regeneration times would then be 40 days and 66 days respectively (adding on the 14 days for the stratum corneum).

The idea of the 28 day epidermal regeneration time seems to have started with the pioneering use of radioactive glycine to study epidermal turnover by Rothberg *et al.* (1961). These authors gave C^{14}-labelled glycine to 2 patients with leukaemia to study normal epidermal turnover and to 2 patients with psoriasis to study the abnormal. Scales from the psoriasis and stratum corneum scraped from the leukaemics every other day were collected, the protein hydrolyzed, and the amount of radioactivity in the glycine determined.

The authors assumed that the glycine would be incorporated into all living cells up to and including the granular layer, but that the greatest incorporation would be in the basal layer where most protein was being formed. If these suppositions were true, then the first appearance of radioactivity at the surface would represent the time needed for a cell to travel from the granular cell layer to the surface (stratum corneum regeneration time), while the appearance of peak activity would represent the time needed for cells to travel from the basal layer to the surface.

As already pointed out, this first assumption was valid and gave a transit time of 14 days for normal stratum corneum and 2 days for psoriasis. They also found a peak of radioactivity in the proteins of the scraped stratum corneum on day 26 in 1 patient and on day 28 in the other, and this was interpreted as the " turnover time " of the normal epidermis. In both instances these are single sharp points having an activity 2 to 3 times greater than all the other specimens examined. In the psoriatic material (2 patients) a rapid rise to a peak at 3 days was found, followed by a gradual decline over the next 9 days.

It is difficult to decide on the meaning of this finding. The injected glycine is incorporated into proteins which are turning over, i.e. being formed and destroyed in the epidermis, as well as into proteins which are stable and which will appear in the surface scale. The level at which epidermal glycine incorporation is maximal is quite uncertain, since Weinstein (1965) found it to be distributed evenly throughout the epidermis by radioautography and Fukuyama and Epstein (1966) found it to be rather high in the epidermis. It is unlikely to be the basal layer, however, since after the injection of radioactive methionine, Porter and

Shuster (1967) found that it appears in the surface scale several days after the peak for glycine. This would imply that methionine is incorporated maximally at some level below the level where glycine is maximally incorporated. Because of these problems I do not think any conclusions as to total epidermal turnover time can be drawn from these studies of stable protein formation in the epidermis.

Van Scott and Ekel (1963) next did an extensive study reconstructing the architecture and mitotic activity of the psoriatic lesion as compared to normal. They concluded that psoriatic epidermis turned over 7 times faster than normal and, *if Rothberg* et al. *were correct in saying that it took* 28 *days for normal epidermis to regenerate, then psoriasis must take only* 4 *days* (my italics). However, as shown above, Rothberg *et al.* were not correct, so although the relative speed of regeneration may be accurate (i.e. 7 times faster than normal), the absolute figure chosen (4 days) was inaccurate. Moreover, in the above study, calculations were based on the fact that there were 27 times as many cells in mitosis per unit area in psoriasis as in normal skin, but the differences in cell cycle (Weinstein and Frost, 1968) were unknown at that time, and these differences would also affect the results.

The first detailed study using tritiated thymidine incorporation into DNA was reported by Epstein and Maibach in 1965. From the labelling index of the germinative cell layers they calculated that the " turnover time " of the germinative cell layers was about 6 days, while renewal of the entire viable epidermis required about 17·7 days. The calculations required knowledge of the DNA synthesis time, and they assumed that 7 h would be in agreement with other tissues, although Sherman *et al.* (1961) had previously shown in mouse ear epidermis that DNA synthesis required 30 h. The actual figure for *in vivo* human epidermal DNA synthesis time, as determined by Weinstein and Frost (1969), is 16 h. If the calculations of Epstein and Maibach are corrected for this error, the respective turnover times of the germinative layer and the viable epidermis become 14 days and 40 days. These figures, combined with the transit time of 14 days through the stratum cornem, gives a total epidermal regeneration time of approximately 54 days.

Of importance in this study was the observation that, unlike the situation in the stratum corneum, the newly-formed cells in the viable epidermis made their way up to the surface in random fashion. This randomizing could be due either to different rates of migration through the epidermis or just to the fact that the cells are released at different times from the germinative cell layer. The first labelled cells to arrive at the granular layer did so in 10 to 14 days, but this is the minimal transit time not the average, and it does not represent the turnover time. Because of the random movement of these cells, the spread between those cells arriving first at the granular layer and those arriving last is quite large, and it is only the mean or average figure which represents a true measure of viable epidermal turnover.

A study of both normal and psoriatic epidermis was reported in 1965 by Weinstein and Van Scott, using similar methods. They confirmed the fact that the *minimal* (my addition) transit time was 12–14 days from germinative cell layer to stratum granulosum in normal epidermis, and approximately 2 days in psoriasis; however, they treated this minimal transit time as if it were the average or renewal time, even although they noted also the random movement of cells from the germinative layer to the granular layer.

DISCUSSION AND CONCLUSIONS

The approximate durations (based on the preceding calculations) of the turnover or renewal times of the various compartments of normal and psoriatic epidermis are given in Table I. Total epidermal renewal is probably between 52 and 75 days in normal skin and between 8 and 10 days in psoriasis. The time needed for a cell to travel to the surface once it has been formed is probably between 40 and 56 days in normal skin and about 6 to 8 days in psoriatic skin. By hindsight it is understandable how the former figures of 28 days for normal and 4 days for psoriasis were derived and why they continue to be used. It is unfortunate, however, that current investigators have been misled by these older figures. Cavill *et al.* (1969), for example, found a disappearance curve for intradermally-injected radioactive iron which had a half-life of about 60 days. Since this was much longer than the " known " turnover time of 28 days for human epidermis, they postulated that the iron must be recycled within the epidermis. Perhaps this speculation is not necessary if the turnover time is really much longer. Even if for no other reason than to avoid misleading others, it is time to reconsider the problem and to lengthen our concept of epidermal turnover time.

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HISTOCHEMICAL APPLICATIONS OF SKIN SURFACE BIOPSY*

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SUMMARY.—The method of skin surface biopsy in which a cyanoacrylate adhesive is used to remove a coherent strip of stratum corneum, has been used to investigate aspects of epidermal function. The Sudan dyes were employed to demonstrate sebum in the hair follicle openings. Although increased quantities of Sudanophilic material were detected with increasing time intervals before sampling, it proved impractical to measure sebum secretion quantitatively by this method. Hydrolase activity in and around hair follicle orifices of the back, forehead and forearm were demonstrated, using enzyme histochemical methods for non-specific esterase, acid phosphatase, leucine aminopeptidase and β-glucuronidase activities. Specimens from the forehead and back were found to have stronger activities than those from the forearm. Apart from the vicinity of the follicular openings, non-specific esterase activity was not normally found in the superficial stratum corneum, although scattered areas of activity were present in the deeper parts of the horny layer. Strong, non-specific esterase activity was detected in skin surface biopsies from lesions of pityriasis lichenoides and, to a lesser extent, those of psoriasis. Mitochondrial enzyme activities were retained in parakeratotic scale from patients with psoriasis, pityriasis rubra pilaris and areas made parakeratotic after repeated removal of the stratum corneum, although not from the lesions of pityriasis lichenoides. The method also proved suitable for the demonstration of melanin granules, blood pigment and sweat gland activity.

THE stratum corneum is functionally the most important part of the skin. It serves as a barrier to the free passage of fluid and protects against microbial and physical injury, i.e. it functions as a membrane in the horizontal plane. Conventional investigative tools examine the structure of the skin at a particular point in vertical cross section and do not allow an overall appreciation of this membrane function of the horny layer.

Recently a method has been described for the examination of the stratum corneum in the horizontal plane, using the cyanoacrylate adhesives (Marks and Dawber, 1971a). These adhesives polymerize rapidly in the presence of moisture and with light pressure to produce a very strong bond with a similar refractive index to glass. This method of " skin surface biopsy " has already been used to investigate the *in situ* microbiology of the stratum corneum (Marks and Dawber, 1971b), and the surface ultra-structure of the horny layer (Dawber, *et al.*, 1971). In this investigation the functions of the stratum corneum have been examined by

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taking skin surface biopsies and employing histochemical techniques to investigate the resulting specimens.

MATERIALS AND METHODS

Skin surface biopsy technique.—The cyanoacrylate adhesive used in this investigation was ethyl cyanoacrylate (" Permabond "—Staident, Staines, Middlesex). A drop of the adhesive was placed on the area of skin to be examined and overlayed with a slightly dampened microscope slide or cover slip. The slide or cover slip was gently pressed on to the drop of cyanoacrylate on the skin for 20–30 s and then removed. An intact layer of horn was removed with the slide, leaving the underlying skin undisturbed. Skin surface biopsies were examined microscopically, mostly in the conventional manner with the coverslip nearest the objective lens. This allowed inspection of the undersurface of the specimen so that epidermal ridges appeared as grooves and vice versa. This could be rectified if required by turning the slide over so that the coverslip was nearest the light source.

Sebum secretion studies.—In order to determine whether the method could be adapted to give a measure of the rate of sebum secretion, the forehead was examined in 15 normal subjects. The forehead was wiped vigorously with ether-soaked swabs and the subject was not allowed to touch this area during the period of examination. A skin surface biopsy was taken from 1 site on the forehead at the start, and further specimens were taken at intervals over a period of 1 or 2 h. The unfixed surface biopsies were then stained with Sudan IV for 15 min or Sudan black at 0°C for 20 min.

Follicular hydrolase activity.—Skin surface biopsies were taken from the forehead, upper back and forearm in 12 individuals, and examined for non-specific esterase activity, using the indoxyl acetate method (Holt, 1954). In 6 of these subjects additional specimens were taken from adjacent areas and tested for acid phosphatase (Barka, 1960), leucine aminopeptidase (using 1-4-methoxy-β-naphthylamide and method of Nachlas *et al.*, 1957), and β-glucuronidase (naphthol AS-BI method of Hayashi *et al.*, 1964).

Enzyme histochemistry of normal and parakeratotic horn.—Skin surface biopsy specimens were taken from the forearms of 6 normal individuals, the lesions of 5 patients with psoriasis, 2 patients with pityriasis rubra pilaris, 3 with pityriasis lichenoides chronica, and from the scaly areas artificially produced after 4 days in normal skin by the repeated removal of stratum corneum with adhesive tape. These were then examined by the following enzyme histochemical methods: non-specific esterase (indoxyl acetate method of Holt, 1954); NADH diaphorase (Pearse, 1960); Cytochrome oxidase (metal chelation method of Burstone, 1959); and glucose-6-phosphate dehydrogenase (Pearse, 1960). In some cases, specimens were examined from the deeper layers of the stratum corneum by repeatedly sampling the same site. Skin surface biopsies from representative sites in these individuals were also stained with H. and E. after brief fixation in absolute alcohol in order to identify nuclei present in the horn.

Examination of stratum corneum for melanin and blood pigments.—Skin surface biopsies were taken from 6 Negroid subjects and from 8 other individuals whose skin colour varied from " fair " to brown. These specimens were stained for melanin with 1% silver nitrate for 1 h and then " fixed " in a 2% sodium thiosulphate solution for 20 min. The Prussian blue reaction was performed on the skin surface biopsies from 6 patients with pigmented stasis dermatitis and on 2 warts showing " black dots " indicative of thrombosed capillaries.

Recording sweat gland activity.—In order to investigate sweating, using the skin surface biopsy technique, the palms of 6 normal subjects were studied. The area to be examined was dried and dusted with quinizarin powder, using the method of Guttman (1941), and the excess was gently blown off. As soon as rows of mauve dots started to appear, the skin surface biopsy was taken from this site.

OBSERVATIONS AND DISCUSSION

Sebum Secretion Studies

The pilosebaceous orifices were outlined by the lipid stains used and the size of the lipid staining area increased with increasing time intervals (Fig. 1). In specimens taken after 30 min there was an increased density of lipid staining material, but in many specimens the areas between the hair follicle mouths were patchily stained as well. The grooves and furrows within the horny layer contained Sudanophilic material before other interfollicular areas, and appeared to act as conduits for sebum. It is interesting to note in this respect that the pilosebaceous openings often served as focal points for the meeting of several of these grooves. Although there was an obvious increasing density of perifollicular Sudanophilic material on skin surface biopsies taken at progressively increasing intervals of time, it was not possible to quantitate this increase. It had been hoped to plot the diameter of the Sudanophilic material around the hair follicle orifices as a function of time, but the existence of the grooves in the stratum corneum made this impracticable. The identification of follicular lipid on skin replicas placed in apposition to the area by staining with osmium tetroxide has been described by Sarkany and Gaylarde (1968).

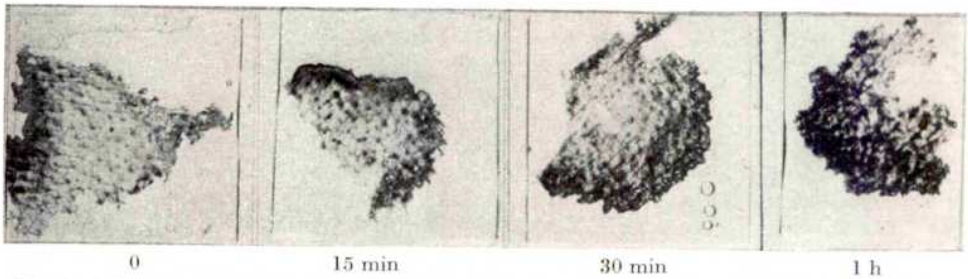

FIG. 1.—Photograph of 4 skin surface biopsies taken from adjacent sites on the forehead before and 15 min, 30 min and 1 h after cleaning the site with ether and staining the specimen with Sudan black.

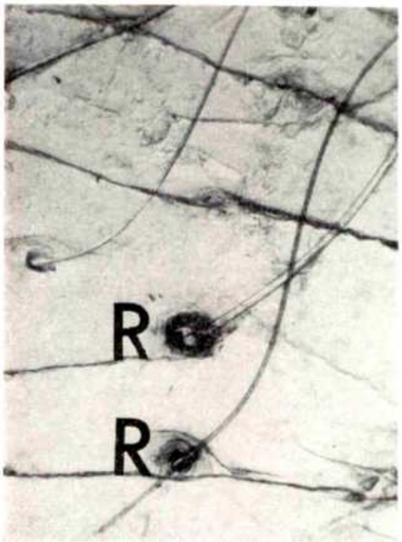

FIG. 2.—Photomicrograph of skin surface biopsy from chest and forearm showing deposition of indigo reaction product as a result of the non-specific esterase reaction around the mouths of hair follicles (× 90). (R indicates perifollicular deposition of reaction product).

Follicular Hydrolase Activity

The blue indigo reaction product of the indoxyl acetate non-specific esterase reaction was located in and around the pilosebaceous orifices (Fig. 2). At times it was possible to see, in avulsed hairs, that the whole of the pilary canal showed non-specific esterase activity. The follicular openings were also positive for acid phosphatase, leucine aminopeptidase and β-glucuronidase. In general the hydrolytic activity was best demonstrated with the non-specific esterase reaction, and the specimens from the forehead and back showed stronger follicular reactivity than the other sites tested. Nicholaides and Wells (1958) noted that surface lipid contains free fatty acids whereas steatocystoma lipid does not. It is implicit in recent thought on acne that an essential step in its pathogenesis is the hydrolysis of the triglycerides of sebum somewhere in the pilosebaceous unit, with the liberation of irritant and comedogenic fatty acids (Kligman *et al.*, 1968).

Follicular hydrolytic activity identified in this study would appear to be unconnected with that usually seen in the normal granular cell layer and the lower stratum corneum; leucine aminopeptidase and β-glucuronidase are not found normally within the epidermis or horny layer at all. The most likely explanation for the presence of lysosomal enzyme activity in and round the hair follicles is that this activity is due to the normal microbial flora in this area. Further work is planned to attempt to relate this hydrolytic activity to the clinical activity of acne.

Histochemistry of Normal and Parakeratotic Horn

Nuclei were easily demonstrated in the parakeratotic horn examined using H. and E. In the parakeratotic horn examined from patients with pityriasis lichenoides chronica, clusters of inflammatory cells were seen amongst the parakeratotic nuclei. Normal stratum corneum did not show any activity for the mitochondrial enzymes. Examination for NAD diaphorase activity of the parakeratotic horn from psoriasis, pityriasis rubra pilaris and from areas made parakeratotic showed a striking perinuclear distribution of formazan reaction products (Fig. 3). This perinuclear pattern was also evident when glucose-6-phosphate dehydrogenase activity was examined. The retention of mitochondrial enzymes in parakeratotic horn probably signifies that the parakeratotic squames are " recent arrivals " from the malpighian layer, and reflects the rapid transit time in psoriasis and pityriasis rubra pilaris. Specimens from lesions of pityriasis lichenoides did not show this striking reactivity. The lack of mitochondrial enzyme reactivity in the parakeratotic scale of pityriasis lichenoides may reflect that the parakeratosis is due to epidermal damage rather than a decreased transit time.

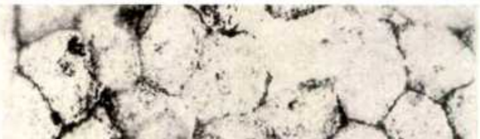

FIG. 3.—Photomicrograph of skin surface biopsies from psoriatic plaques. The NAD diaphorase reaction has been performed, and it is seen that there is perinuclear deposition of the formazan reaction products (× 150).

Non specific esterase activity was normally found perifollicularly (see above) but not elsewhere in the upper part of the normal stratum corneum. When repeated skin surface biopsies were taken from an identical site, it became evident that specimens from the lower part of the stratum corneum contained scattered areas showing some non-specific esterase activity. Specimens from psoriatic plaques showed patchy, non specific esterase activity. There was similar, but more intense, activity in skin surface biopsies from the lesions of pityriasis lichenoides chronica. There was no tendency for a perinuclear distribution of the indigo reaction product, as there tended to be with mitochondrial enzymes.

Further investigations are under way to explore the relationship between the histochemical findings in skin surface biopsies and the metabolic characteristics of the epidermis in the scaling disorders.

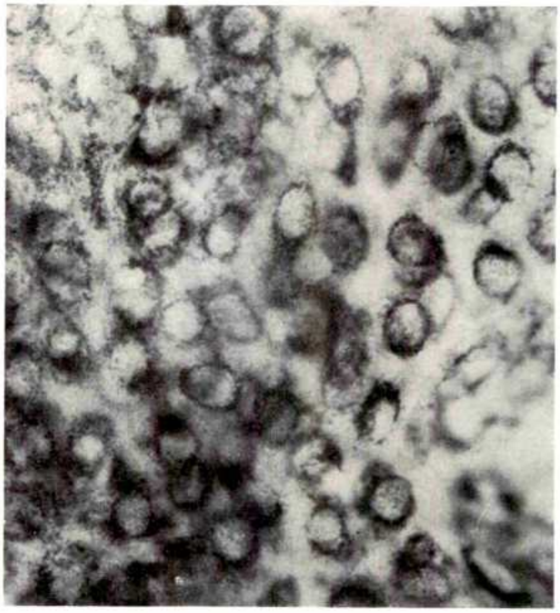

FIG. 4.—Photomicrograph of skin surface biopsy from forearm of a negroid subject; stained with silver nitrate to demonstrate melanin. Numerous melanin granules can be seen within the horny squames (× 150).

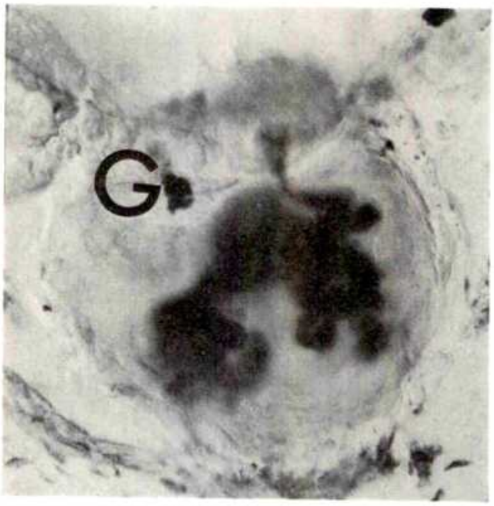

FIG. 5.—Photomicrograph of skin surface biopsy taken from a virus wart showing " black dots ". The Prussian blue reaction has been performed and positive reacting granules (G) can be seen near the " cast off " thrombosed papillary capillary (× 150).

Examination of Stratum Corneum for Melanin and Blood Pigment

Melanin.—Melanin granules were easily demonstrated in all the skin surface biopsies treated with silver nitrate (Fig. 4). Keddie and Sakai (1965) similarly identified melanin granules in stratum corneum removed with Scotch tape. As expected, the number of melanin particles increased with the degree of racial pigmentation. All the melanin granules observed were intracellular and appeared regular in shape and of a similar size.

Blood pigment.—Blue granular deposits, indicating a positive reaction for haemosiderin, were detected in the specimens of 4 of the 6 patients with pigmented stasis dermatitis. In addition, blue staining granular material was detected around the "ghost" of the thrombosed capillary in the virus warts examined. The blood pigment particles, in contrast to melanin, were extracellular, and were irregular in shape and size (Fig. 5).

The demonstration and differentiation of these pigments finds application in 2 clinical situations:

(a) Differentiating pigmentary incontinence from increased pigment production, as only in the latter instance will the horny squames show increased pigment content.

(b) Differentiating black lesions due to the presence of altered blood pigment from those due to increased melanization.

Recording Sweat Gland Activity

The sweat gland orifices were easily seen, using the quinizarin method. The method employing silver nitrate and photographic developer used by Sarkany and Gaylarde (1968) on replicas did not yield as good results with skin surface biopsies. This technique can provide a permanent record of sweat gland activity and hence is useful in charting the effectiveness of therapy for hyperhidrosis or determining the extent of a neurological lesion.

COMMENT

Examination of the stratum corneum in the horizontal dimension is clearly capable of yielding considerable information of use both from the clinical and the investigative standpoints. It has been shown that the skin surface biopsy technique can be used to demonstrate sebum, sweat and melanin production. It has, in addition, been used to examine the enzyme histochemical characteristics of the stratum corneum, and this finds particular application in the examination of parakeratotic horn and in tracing the hydrolytic activity in and around hair follicles. An advantage of the method used in this investigation as compared to a replica technique is that the stratum corneum itself is under examination, so that its properties can be investigated. In addition, the horny layer can be examined and investigated while retaining its *in vivo* inter-relationsips.

The author is extremely grateful to B. Bhogul and N. Ramnarain for valuable technical assistance. The author is in receipt of a grant from the Medical Research Council.

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ULTRASTRUCTURE OF ADULT EPIDERMAL CELLS IN MONOLAYER CULTURE

HILARY CONSTABLE

SUMMARY.—Epithelial cells from adult guinea-pig and human skin were grown in monolayer culture and studied by phase contrast and electron microscopy. Significant differences were found between cells *in vivo* and *in vitro*.

Keratinocytes show a selective dedifferentiation. They retain the ability to make desmosomes, but interdigitation is reduced and the system of tonofilaments is disorganized. Mitochondria become long and even branched while microtubules become prominent, as do lysosomes and fat droplets. Ribosomes often become arranged in polyribosomes, and channels appear in the nucleus. Melanocytes showed similar changes, and 1 produced a cilium.

Such changes should be taken into consideration when cells in dispersed culture are used experimentally.

It is generally recognized that cells in primary culture display characters differing from those *in vivo*, but few detailed ultrastructural studies have been made of cells in tissue culture. Cireli (1970) observed fibroblasts in culture; Erlandson (1971) studied the ultrastructure of synchronized HeLa cells; Pugh-Humphreys (1970) described the ultrastructural qualities of the surface of Landschutz ascites, fibroblastic HeLa cells and canine kidney cells.

In skin, ultrastructural changes in organ culture have been studied by Prose *et al.* (1967) and by Sarkany and Gaylarde (1970). Gazzalo and Prunieras (1968) reported acid phosphatase activity in melanin-containing vesicles *in vitro*. Prunieras (1969) thoroughly reviewed interactions between keratinocytes and dendritic cells both *in vivo* and *in vitro*, but did not mention the differences in ultrastructure seen between the cells in these conditions.

One problem in working with established cell lines, particularly of abnormal cells, is the difficulty in knowing what are immediate adaptations to culture and what are long term changes in the cell line. This study was undertaken to investigate any changes which might take place in normal adult skin cells in primary culture.

MATERIALS AND METHODS

Culture Methods

The cells were obtained and cultured essentially by the method of Cruickshank *et al.* (1960). Thin slices of adult human skin or guinea-pig (*c* 300 g) ear skin were trypsinized, dermis and epidermis separated and epidermis teased to produce a suspension of cells. The suspension of cells obtained was transferred to a 30 ml Falcon flask containing 5 ml of a medium consisting of 60% minimal essential medium (Flow Laboratories)

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buffered with Hepes (Sigma London Chemical Company) (Fisk and Pathak, 1969) and 40% guinea-pig serum, and incubated at 37°C. The medium was changed every 3–4 days. Cells examined were guinea-pig epithelial cells grown for 7, 8 and 12 days and mixed cells from human skin grown for 18 days. Since the ultrastructure of fibroblasts in culture has been described (Cireli, 1970), epidermal cells only will be described here.

Electron Microscopic Methods

Cells were prepared for electron microscopy, using a modification of the method of Brinkley *et al.* (1967). The monolayer was fixed *in situ* by decanting the culture medium and replacing with ice-cold 5% glutaraldehyde in 0·2 mol/l cacodylate buffer for 30 min, washed 3 times with 0·2 mol/l cacodylate buffer for at least 5 min and post fixed in 2% osmium in 0·2 mol/l cacodylate buffer for 15 min. The flasks were then dehydrated in 50%, 70%, 85%, 95% and 2 changes of absolute ethanol for 15 min each. Epon was introduced through the neck of the flask after the second change of absolute alcohol was removed.

The top of the flask was then removed with a hot scalpel, leaving the sides, and then 2 more changes of Epon were made at ½–1 h intervals. The Epon was then poured off, leaving a layer approximately as thin as a coverslip. The Epon was polymerized for 36 h at 60°C. Such a thin layer of Epon allowed the cells to be viewed by phase contrast after embedding. The sides of the flask were then removed with a hot scalpel, and groups of cells were selected using phase contrast microscopy and circled with a Leitz diamond objective marker.

The selected cells were then obtained by 1 of 2 methods. A mechanical cork borer applied to the Epon was used to obtain a disc of plastic and Epon. When these were removed from the cork borer they were usually easily separated. In some instances the flask was cooled to −20°C and broken; the layer of Epon was peeled off and the circles clipped out with fine scissors. Both methods sometimes produced stress marks and made it difficult to see the cells, but it was still possible to take satisfactory sections from these pieces. The pieces of Epon obtained were then mounted on pre-polymerized blocks of Epon or Araldite with commercial Araldite which was allowed to polymerize at room temperature. The pieces of Epon were mounted so that the cells were at the surface; this was to avoid cutting through a variable thickness of Epon before coming to the cells.

The face of the block was trimmed with razor blades to a pyramidal shape, and ultra-thin sections cut immediately. Thick sections for light examination were not taken.

Sections were picked up on uncoated copper grids, stained with saturated methanolic uranyl acetate and viewed in a Siemens Elmiskop 1.

RESULTS

When epidermal cells are dispersed with trypsin and cultured in monolayer, a mixture of keratinocytes and melanocytes usually results. Several phase contrast microscope studies have been made (Cruickshank *et al.*, 1960; Cruickshank and Harcourt, 1964; Cohen and Szabo, 1968) which adequately describe the cells as seen by light microscopy. The keratinocytes, which are usually assumed to be from the lower layers of the epidermis, grow in typical spreading groups which may merge to give a confluent sheet. In monolayer cultures the melanocytes are easily distinguished by phase contrast microscopy. Their long dendrites reach out in several directions and may make contact with more than one keratinocyte.

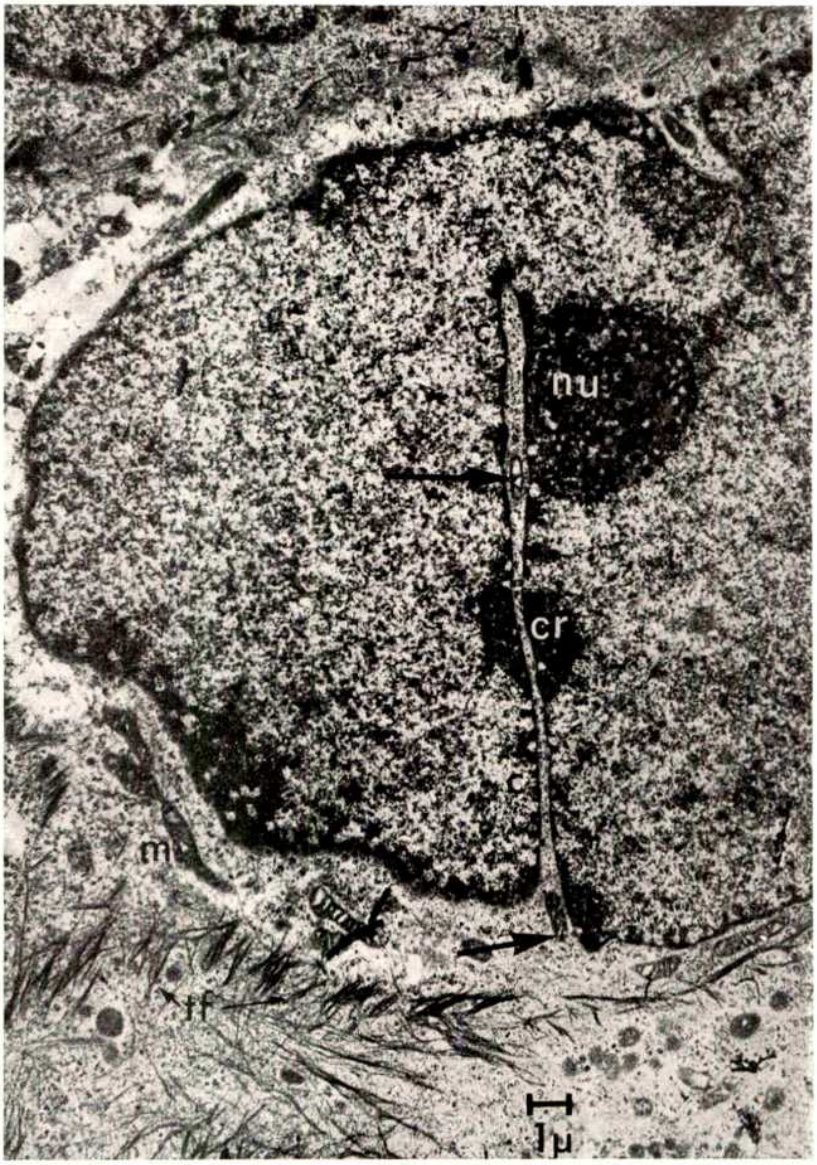

FIG. 1.—Keratinocyte with a channel (c) in the nucleus and associated mitochondria (arrows). Nucleolus (nu), chromatin (cr), dispersed tonofilaments (tf), branched elongated mitochondrion (m).

Ultrastructure of Keratinocytes

In cultures of keratinocytes the nuclei are round or ovoid and are " higher " than the thickness of the surrounding cytoplasm so that the cell in culture is like a fried egg. This is shown by the finding that some sections showed only a nucleus surrounded by a thin rim of cytoplasm. The nuclei often have long thin clefts or channels reaching some way across them. These channels, which sometimes branch, are consistently about 0·5 μm wide and bordered with dense chromatin. Such channels are not seen in whole skin, where basal keratinocytes have a round or ovoid nucleus with characteristically little indentation. Mitochondria are often seen in or at the mouth of these channels (Fig. 1).

In monolayer the mitochondria become dense and elongated, reaching as much as 5 μm, although they remain 0·3 μm wide, as in whole skin. In culture they are often branched in contrast with the near spherical ones of whole skin (0·3 μm × 0·3–0·7 μm) (Fig. 2). Many cells showed prominent Golgi areas (Fig. 3).

In these keratinocytes many sections show large numbers of polyribosomes not apparent in whole skin (Fig. 3, 4, 5). Large secondary lysosomes (0·5 μm) packed with membranes (Fig. 6) and also fat droplets (Fig. 2, 3) are seen in cultured cells and are sometimes very numerous, contrasting with normal whole skin where they are seldom seen.

In monolayer, microtubules are very prominent and sometimes traverse the cytoplasm for long distances (Fig. 2, 4, 5, 7).

In cultured keratinocytes and in whole skin there are a variety of cell junctions. In confluent monolayer there is often a substantial intercellular space of about 0·6 μm (Fig. 5). In other areas the plasma membranes lie closely apposed (Fig. 4). Interdigitations, although present, are much less frequent than in whole skin (Fig. 2). In cultured cells, desmosomes with their immediately associated tonofilaments are present and normal in structure (Fig. 5), but the rest of the tonofilament network appears disorganized (Fig. 7), in some cases presenting an appearance similar to that seen in acantholytic cells. The tonofilaments are often distributed very unevenly through the keratinocyte, some parts of which are devoid of tonofilaments, while these are present elsewhere in the same cell (Fig. 3, 4), contrasting with the regular orientation of tonofilaments in whole skin (Zelickson, 1971). In some areas the membrane was distinct, showing normal ultrastructural features; elsewhere the border of the cells was very indistinct and attenuated, perhaps representing ruffles, as seen by time-lapse cinemicrography. Melanosomes are present, scattered throughout the cytoplasm, usually in membrane-bound groups, as described by Gazzalo and Prunieras (1968). Keratinosomes and kerato-hyaline granules were not seen.

Ultrastructure of Melanocytes

There were fewer melanocytes than keratinocytes in these cultures and the sections often showed dendrites but not the body of the cell. The nucleus of 1 cell observed had a narrow channel and associated mitochondrion, similar to those described in keratinocytes. A most unusual finding in this same melanocyte was a cilium. The proximal end of this cilium was seen projecting from the cytoplasm through the plasma membrane. The cilium was aligned with the channel in the nucleus (Fig. 8a).

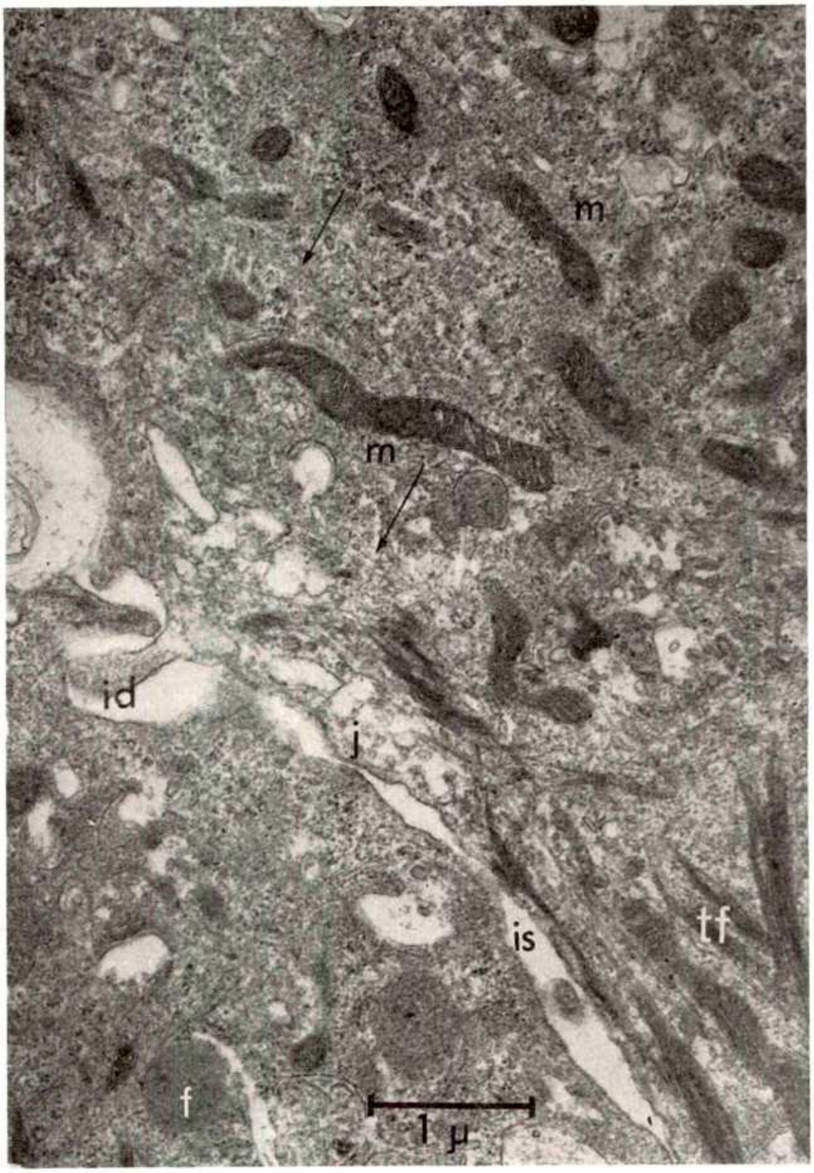

FIG. 2.—Keratinocytes showing intercellular space (is), interdigitations (id), and close junctions (j). Within the keratinocytes are mitochondria (m), microtubules (arrows), a fat droplet (f) and tonofilaments (tf).

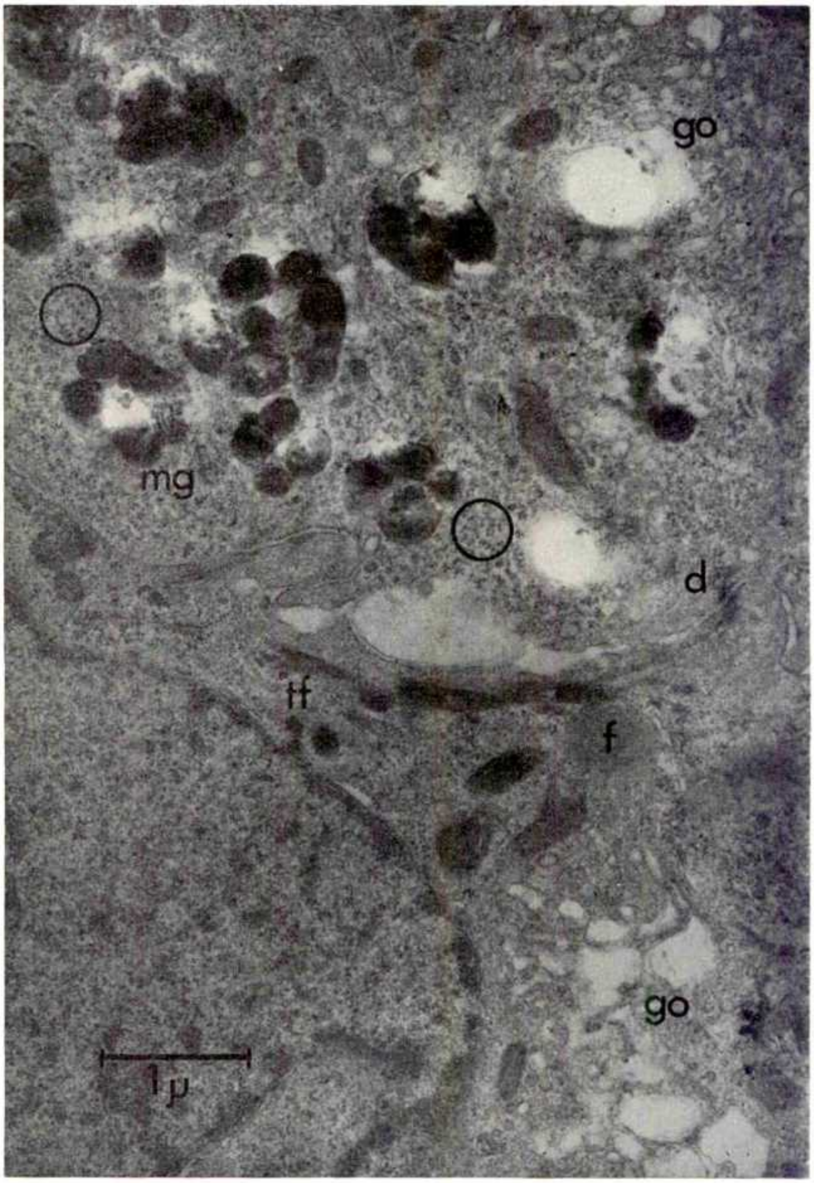

FIG. 3.—Two keratinocytes joined by a desmosome (d). Note the lack of tonofilaments in the remainder of the upper cell. Both cells have a prominent Golgi apparatus (go). Polyribosomes (circles), melanin granules (mg), tonofilaments (tf), fat droplet (f).

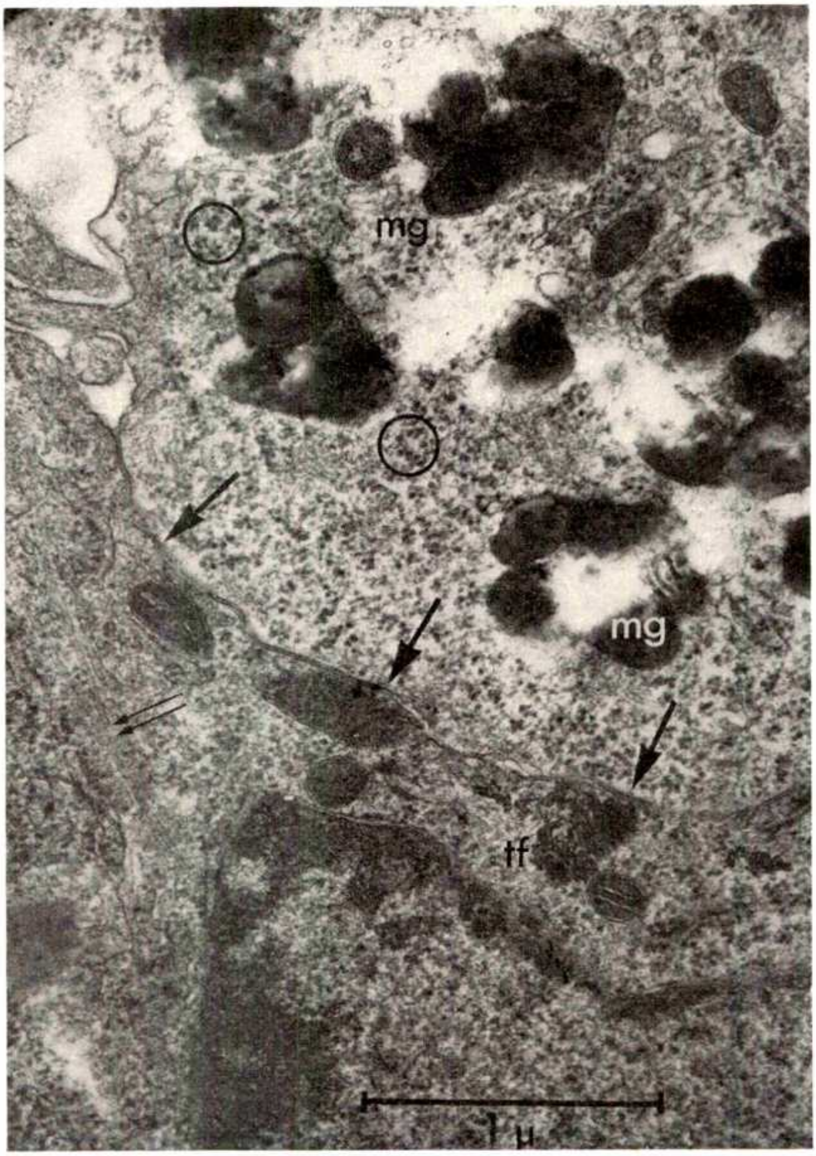

FIG. 4.—Another section of the same 2 keratinocytes. Note close apposition over some distance (arrows). Melanin granules (mg), microtubules (double arrow), dispersed tonofilaments (tf), polyribosomes (circles).

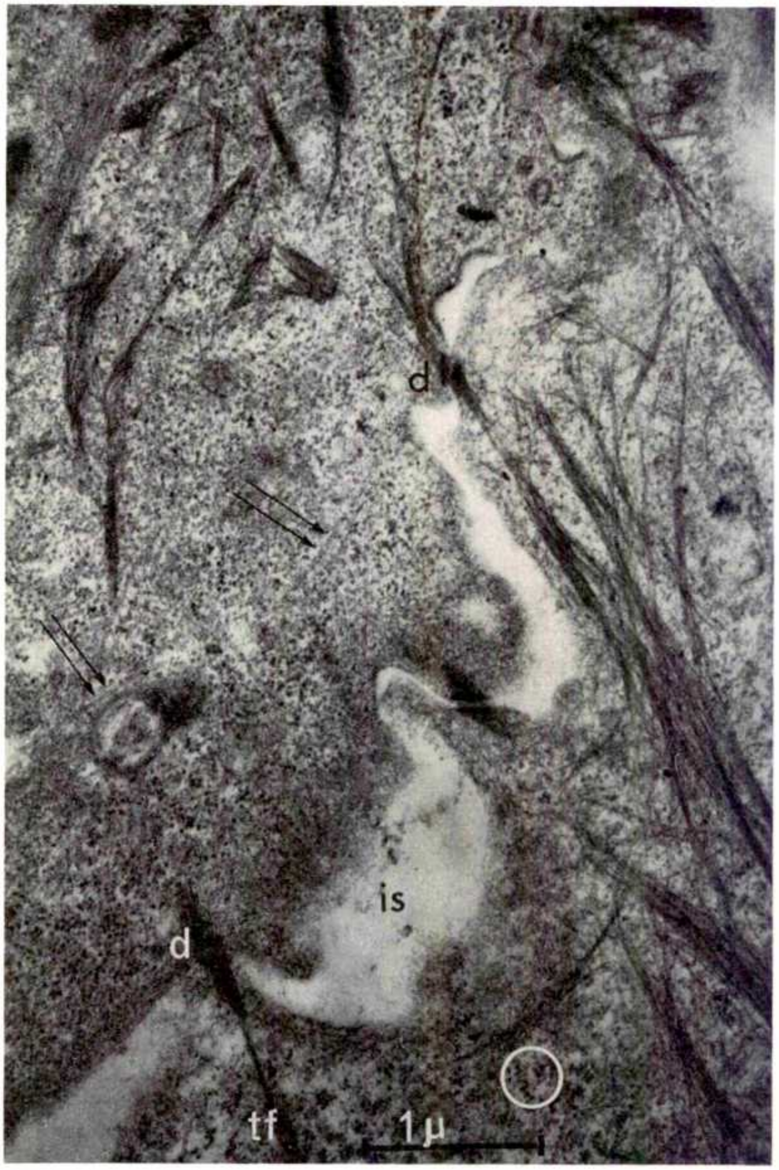

FIG. 5.—Keratinocytes with intercellular space (is), desmosomes (d), and associated tonofilaments (tf). Microtubules (double arrows) and polyribosomes (circle).

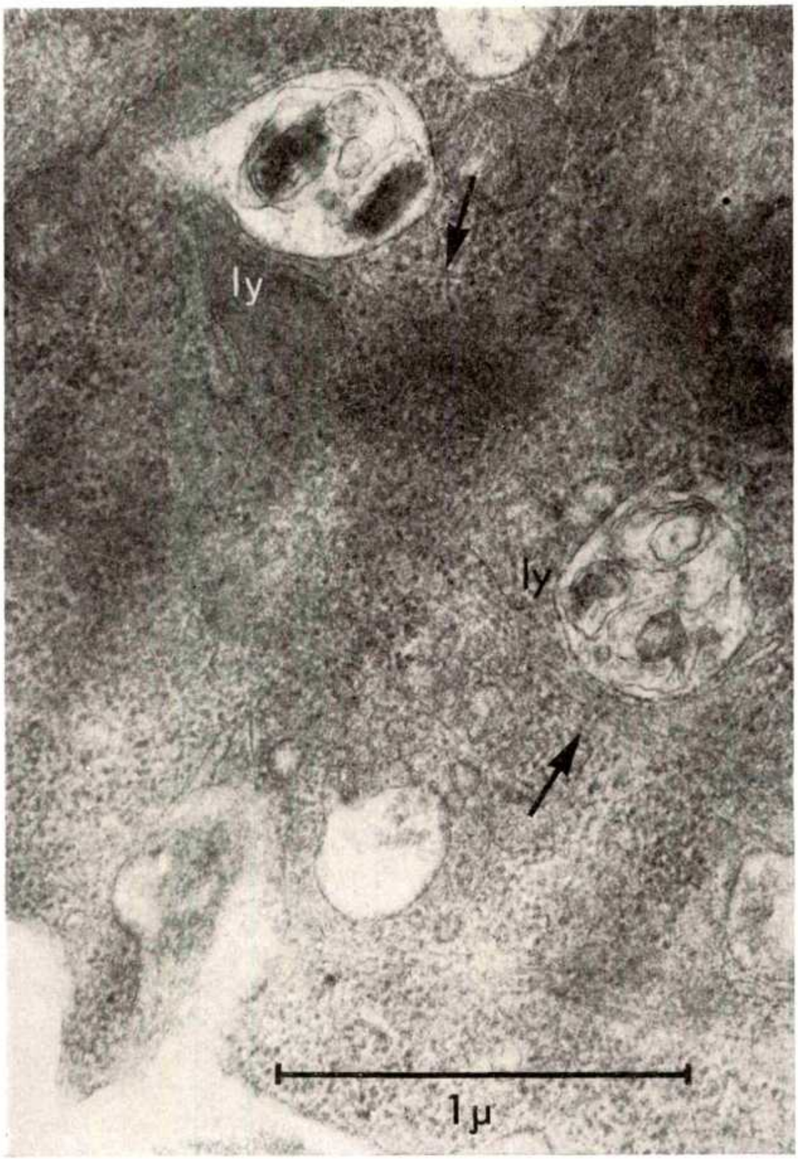

FIG. 6.—Keratinocytes with large secondary lysosomes (ly) and microtubules (arrows).

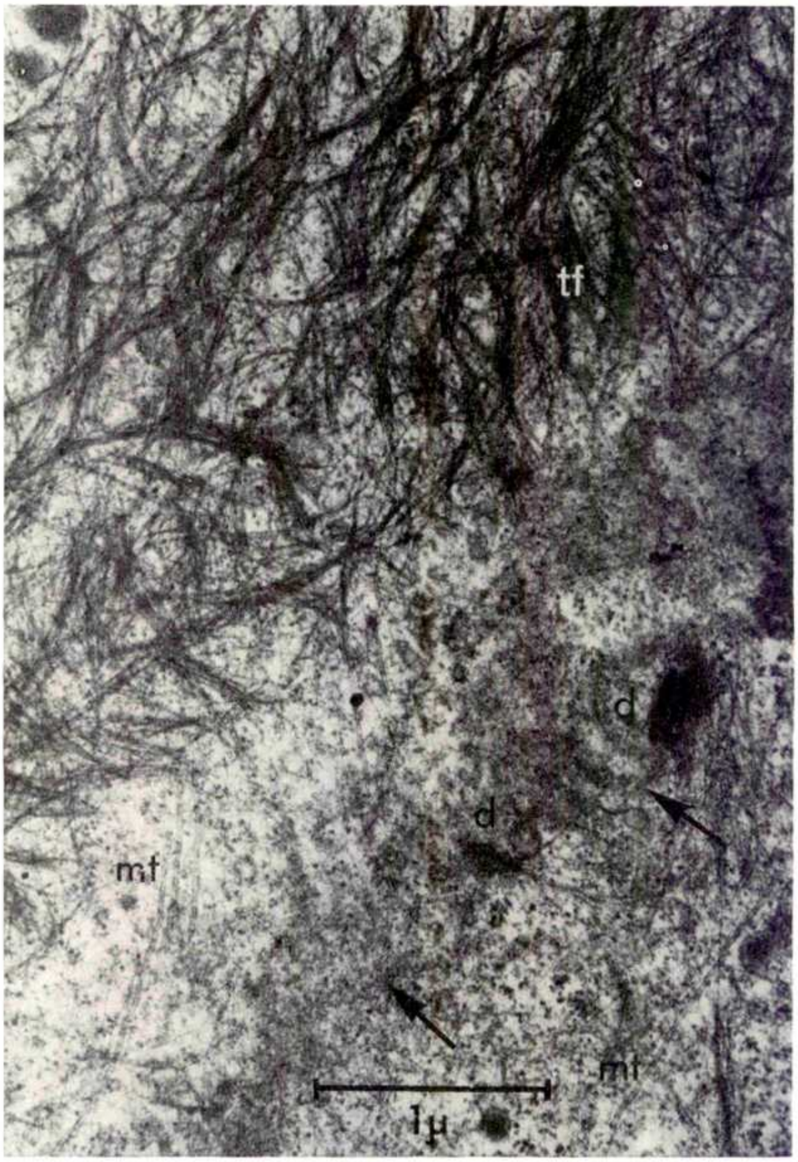

FIG. 7.—Keratinocytes showing very indistinct boundaries (arrows). Desmosomes (d), prominent microtubules (mt) and disorganized tonofilaments (tf).

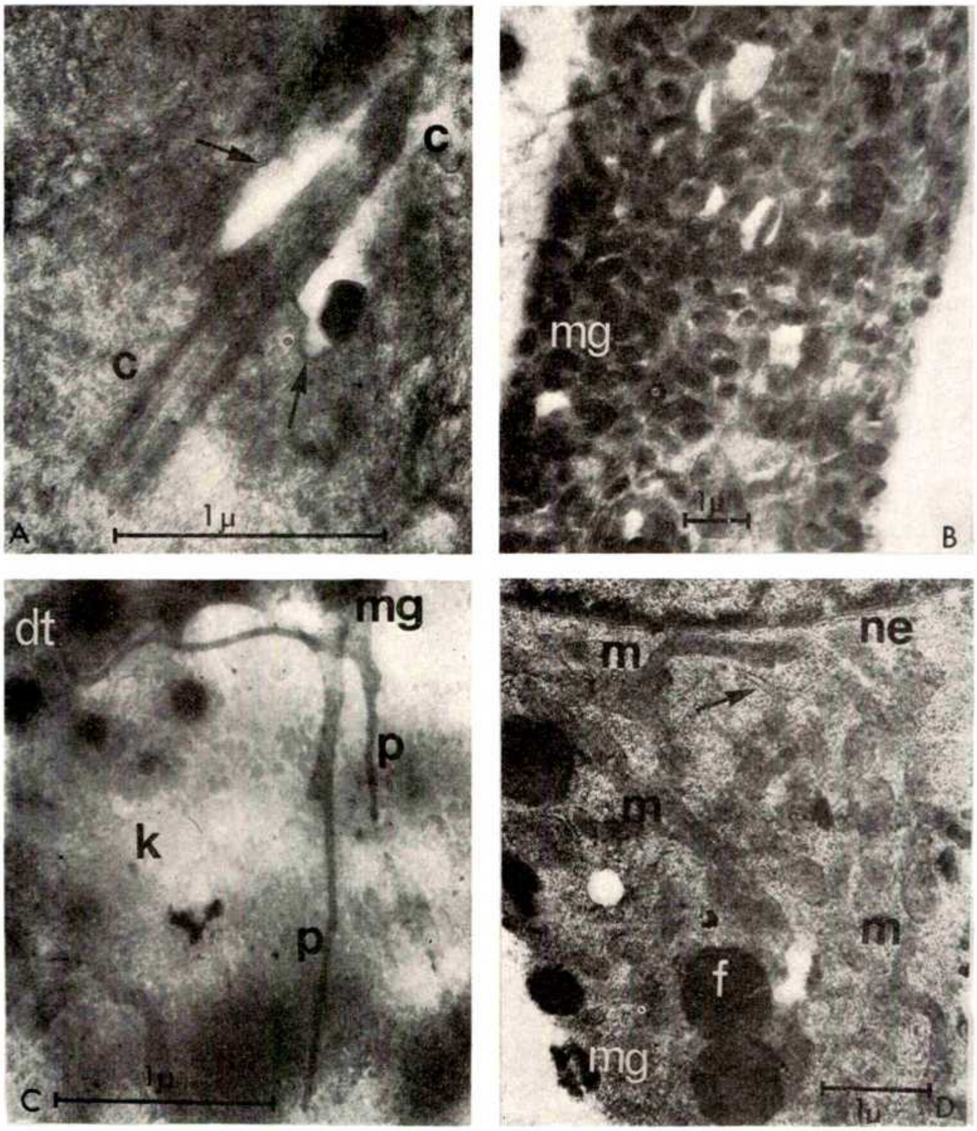

FIG. 8.—Melanocytes in culture. 8a.—Cilium (c) projecting through plasma membrane (arrows). 8b.—Melanocyte dendrite packed with melanin granules (mg), to the exclusion of other organelles. 8c.—Fine processes (p) from melanocyte dendrite (dt) extend over keratinocyte (k). Melanin granule (mg). 8d.—Part of a melanocyte with few melanin granules (mg), showing changes as seen in keratinocytes. Fat droplets (f), elongated and branched mitochondria (m), nuclear envelope (ne), and rough endoplasmic reticulum (arrow) are visible.

The distribution of melanosomes was essentially similar to that seen in whole skin. Some dendrites were packed with melanosomes to the exclusion of other organelles (Fig. 8b), whereas others were almost devoid of them. The tips of many dendrites had long thin extensions, which must have been invisible by light microscopy, and it was often difficult to establish whether they penetrated other cells or overlaid them (Fig. 8c). Membranous flaps also extended from the sides of some dendrites and over adjacent cells.

The usual cell organelles were present in melanocytes and, as in the cultured keratinocytes, the mitochondria were elongated and branching (Fig. 8d); fine fibrils and microtubules were also seen.

DISCUSSION

The present study has shown that epidermal cells in tissue culture retain some of the characteristics which they have *in vivo*, lose some, and develop other new features. Changes seen must be a reflection of the altered conditions in which the cells find themselves. Such factors may be the changes in the nutritional status, the absence of the influence of other cells and structures to which they are exposed in the normal anatomical tissue relationship, and the nature of the surface upon which they are growing. There is little doubt also that their rate of replication is considerably increased in monolayer culture.

It will be noted that the keratinocytes contain neither keratinosomes nor keratohyaline granules. This is consistent with the concept that they are derived from cells of the basal layer of the epithelium, and it would seem that they are unable to develop these structures or show any sign of keratinization in culture. It may be that the cells are not unable to keratinize but are lacking the instruction needed to start the process—perhaps an increase or decrease of some substance. In whole skin the complexity of intra-epidermal and dermo-epidermal reactions has been well discussed (Billingham and Silvers, 1963; Fleischmayer and Billingham, 1968) and cultures of dispersed keratinocytes lack such interactions, having neither dermal elements nor basal lamina. It may be that the cells are producing substances without instructions for their further metabolism or use, and accumulations of fat droplets and the development of lysosomes may occur as a result of this.

Desmosomes were very prominent as much as 12 days after starting culture, and the absence of the half desmosomes caused by initial trypsinization (personal observation) indicates that these are formed *de novo* in culture. Further evidence for the retained ability to form desmosomes is that the number of new cells formed by several divisions greatly exceeds the numbers of initially viable cells. Continued presence of desmosomes and tonofilaments indicates that dedifferentiation is selective. It has not so far been possible to show how the desmosomes are formed. Sequential studies at short intervals after setting up the cultures should help to resolve this question.

The altered arrangement of the tonofilaments and irregular distribution is likely to be due to their altered spatial relationship allowing contact in only 1 plane. Growth in 1 plane and the lack of the normal tension and abrasion to which skin is subjected would also tend to minimize the necessity for support in 3 dimensions afforded by interdigitations.

The cells in these monolayer cultures are spread flat and embedded so that the sections are tangential. In this way most organelles are likely to be parallel to the plane of the section and so thus be easily seen. This makes it difficult to make a numerical comparison with the cells *in vivo*.

The enlarged mitochondria indicate a raised rate of respiration in tissue culture, which is not unexpected when cells are actively growing and dividing. Studies are being undertaken to measure this under normal and experimental conditions. The numerous polyribosomes indicate altered protein synthesis.

One of the characteristics of epidermal cells in culture would seem to be the development of prominent microtubular systems. Microtubules are said to be associated with mitosis, axonal flow and melanin granule movement, and to have a skeletal function in platelets (Soifer, 1971). Their precise role in cultured skin cells has not so far been ascertained, but these cells have a greatly increased mitotic rate and are also relatively motile.

It is difficult to explain the development of a cilium in a culture of adult skin. Stubblefield and Brinkley (1966) found cilia in hamster fibroblast cultures treated with colcemid, which is known to interfere with microtubules. Cireli (1970), in his electron microscopic studies of monolayer cultures of human fibroblasts, found a cilium having the same appearance as that described here. Cilia have been described in various foetal human ectodermal and epidermal cells (Breathnach, 1971). It may be that the situation of somewhat dedifferentiated cells in a fluid environment has some similarity to the conditions of foetal cells in amniotic fluid. Clearly there are some fundamental changes in the metabolic balance of these cells in culture if such a highly differentiated and specialized cell as a melanocyte has produced a cilium.

I would like to thank Miss Susan Bradshaw for assisting me with the tissue cultures, and both Dr C. N. D. Cruickshank and Mrs Patricia R. Mann for helpful advice, criticism and encouragement.

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EXPERIMENTAL ZOSTERIFORM HERPES SIMPLEX VIRUS INFECTION IN MOUSE SKIN

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SUMMARY.—A highly reproducible model in which linear zosteriform lesions are produced in the skin of Webster Schneider mice, using an oral strain of herpes simplex virus, (HSV) is described. The incidence of infection is high, and a large proportion of infected animals develop a highly characteristic ascending paralysis which is invariably fatal.

In 1923 unilateral zosteriform herpes simplex infection (HSV) was produced in guinea-pigs by inoculation and scarification after shaving and repeated applications of coal tar had rendered the skin hyperplastic (Teague and Goodpasture, 1923). Tanaka and Southam (1965), using intradermal inoculation, produced comparable lesions in newborn rats, but they failed to achieve lesions once the animals were more than 9 days old and no lesions were noted in newborn mice in similar experiments. In 1964 Force *et al.*, reported that a highly reproducible self-limiting infection can be produced in rabbit skin by the simultaneous inoculation of HSV and 1% hyaluronidase. In 1967 Griffin produced vesicular lesions in the rabbit lip, using a multiple puncture technique, and Tomlinson and MacCallum (1968) induced vesicular lesions in guinea-pigs, which might progress to hind leg paralysis and death unless animals were given antiherpes serum.

Adervont (1929) inoculated the abdominal skin of mice and showed that wide dissemination of the virus followed, and Evans *et al.* (1950) showed that a fatal encephalitis developed in mice when HSV was inoculated into abdominal skin. In 1965 Sydiskis and Schultz inoculated Swiss mice intracutaneously with HSV after epilation of the hair of the flank, and produced zosteriform lesions. They suggested that the incidence of lesions was dose dependent, but that the size and rate of development was independent of dose, and that the mortality of mice with lesions never exceeded 61%. The incidence of lesions was independent of age in mice between 5 weeks and 5 months of age, but the mortality fell with increasing age. They noted vesicular lesions by the third day after inoculation, which lesions soon became necrotic; and, by the fifth post-infective day, a unilateral necrotic band extended from the dorsal to the ventral mid-line on the infected side. In animals that survived the lesions dried up considerably by the tenth post-infective day; and by day 18 almost complete regression of the lesion had occurred. Constantine *et al.* (1971) have produced similar lesions in hairless mice, and their ultramicroscopical studies suggested that viral replication occurred within Schwann cell nuclei and travelled centrifugally in the Schwann cell cytoplasm to produce the characteristic zosteriform lesion.

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Our preliminary studies showed that the incidence of infection depended on the strain and titre of virus, and on the strain and age of mice. Thus, the HFEM strain and 1 oral strain of HSV isolated in our laboratory failed to produce recognizable infection; and if the titre of our stock virus fell below 10^{5-6} TCD_{50}/ml, we noticed an immediate fall in the incidence of mouse skin infection. Four strains of male Swiss white mice (Swiss TO, Tuck No. 1, Porton, and Webster Schneider) were infected initially, and these studies demonstrated conclusively that the Webster Schneider strain was most suitable in our hands: they provided the highest rate of infection, which was consistently reproducible, with easily recognizable skin lesions in the absence of fighting. Multiple bites can produce non-specific lesions indistinguishable from the early zosteriform HSV skin lesion.

This paper reports a highly reproducible model in which Webster Schneider white mice aged 6–8 weeks were infected intracutaneously with herpes simplex virus under ether anaesthesia; linear zosteriform lesions develop by the fourth to sixth post-infective day in a high percentage of animals. The incidence and mortality of infection has been recorded, and studies on the viral growth and histological changes of lesions in the skin and neutralizing antibody titre in the serum have been undertaken.

MATERIALS AND METHODS

Virus.—An oral strain of HSV isolated from a patient at University College Hospital was selected as the stock virus. The virus was grown and passaged in HeLa monolayers, and viral stocks with a titre of $10^{5-6}TCD_{50}$/ml were obtained and stored at −60° C; regular weekly checks on the titre of stored virus were carried out.

Mice.—Male Webster Schneider white mice aged 6–8 weeks (25 g), provided by a single breeder, were used in all experiments. Animals from each weaning group were kept separately, as this virtually eliminated fighting.

Infection of mice.—A band of hair 1·5 cm wide extending from the dorsal to the ventral mid-line was removed from the left flank of each animal under ether anaesthesia. Within 30 min 0·05 ml of undiluted stock virus containing approximately 10^4TCD_{50} was inoculated under ether anaesthesia with a 25 gauge needle. The skin was gripped between curved, plastic-covered forceps, and the needle was inserted at an acute angle so superficially that it could be seen throughout its length within the skin; a bleb was then raised which persisted for about 30 min.

Histological studies.—Mouse skin specimens were fixed in 70% alcohol, processed in the usual way for sectioning, and stained with HE.

Virus assay.—Infectivity titres of HSV were performed in HEp 2 monolayers in T/C tubes, and titres were calculated from the observed cytopathogenic effect, using the method of Reed and Muench (1938). Skin from groups of 5 mice was collected at intervals after infection, homogenized (10% w/v in tissue culture medium), frozen and thawed 3 times, centrifuged at 3,000 rpm for 10 min at 4° C, and the supernatant similarly assayed for viral content.

Antibody assay.—Sera were collected from groups of 5 animals at intervals after infection, and antibody levels were assessed by neutralization, using HEp 2 cells.

RESULTS

Groups of mice were inoculated intracutaneously with HSV, and observations were made on (1) the clinical aspects of the infection (skin lesions, paralysis and death); (2) the interval between inoculation and appearance of lesions, paralysis and death; the total incidence of infection and paralysis and the final mortality rate; (3) the histological changes found at various stages of the skin infection; (4) virus growth in the skin, and serum antibody content.

(1) *Clinical Aspects of the Infection*

Unequivocal skin infection became recognizable from the fourth to sixth post-infective days by the formation of crusted linear zosteriform lesions, which extended around the left flank of infected animals, usually starting at the site of inoculation and spreading up and down to the dorsal and ventral mid-line. Early crusted lesions were discrete, but within 24 h had become confluent (Fig. 1a); and frequently considerable necrosis was evident by the next day (Fig. 1b). Inoculation at the dorsal mid-line produced comparable bilateral lesions completely encircling the animal. Vesicular lesions on about the fourth post-infective day were only occasionally noted. The majority of animals then developed paralysis and died; but in a small proportion the infection was arrested, and the skin lesions healed by the eighteenth post-infective day.

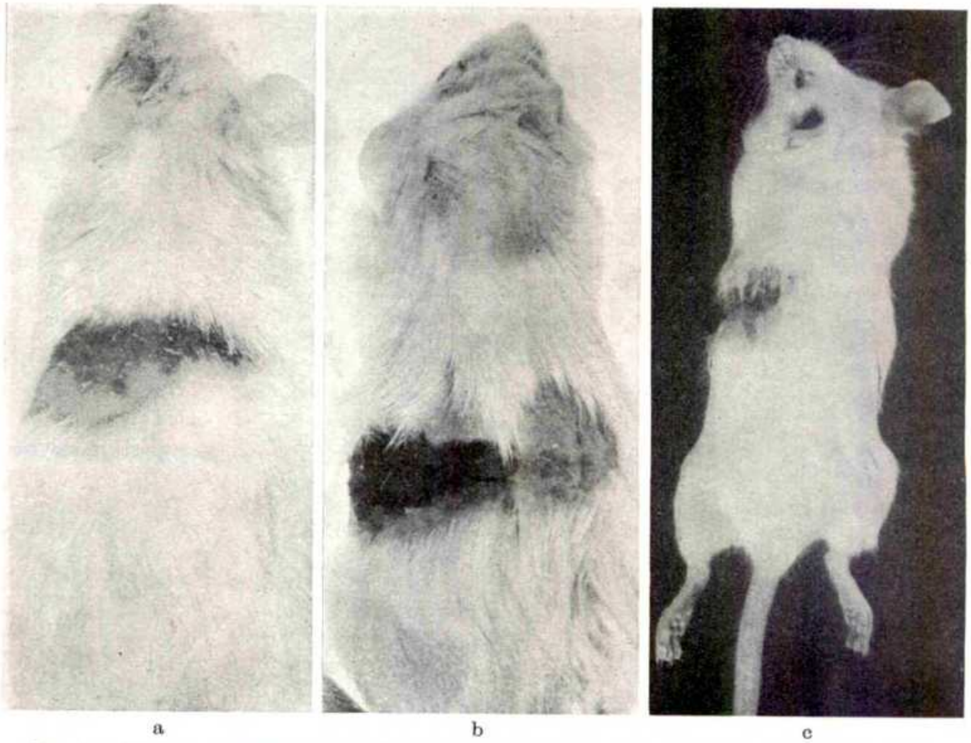

FIG. 1.—Clinical appearances of early skin lesion (a); later necrotic lesion (b); characteristic ascending paralysis (c).

Most animals developed a highly distinctive paralysis, commencing peripherally in the foot on the infected side, progressing to total paralysis of this leg, then the right hind leg, and ascending to complete paralysis and death. Animals almost invariably died in a characteristic position, with both legs extended and the left side of the head uppermost (Fig. 1c).

(2) *Infective Rate, Paralysis, Mortality and Recovery*

Table I contains results from a typical experiment, and the numbers represent the total daily counts of infected, paralysed, dead and recovering animals. It can be seen that the infection becomes manifest between the fourth and sixth post-infective days. Paralysis usually appeared 48–96 h after evidence of infection, and these animals then usually perished within 48 h. Just a few animals that did not become paralyzed recovered completely.

In Fig. 2, histograms represent the percentage of animals (of the total) developing lesions and paralysis, and the number that died on each day. The curves represent the cumulative totals derived from these numbers. More than 50% of animals developed lesions on the fifth day after inoculation with about 15% on the preceding and subsequent days, and a few on the seventh and eighth days; just over 90% of animals in all became infected. Paralysis appeared on significant numbers over a longer period, but the maximum number was noted between days 7 and 9, and about 50% of animals became paralysed. Animals perished mainly between the eighth and thirteenth post-inoculation day, and the total number that died was in the region of 80%. In these experiments, all animals that developed paralysis died, but some animals died that had not been recorded as paralysed; the majority of these, however, were found after death in the characteristic position that suggested paralysis had in fact preceded death.

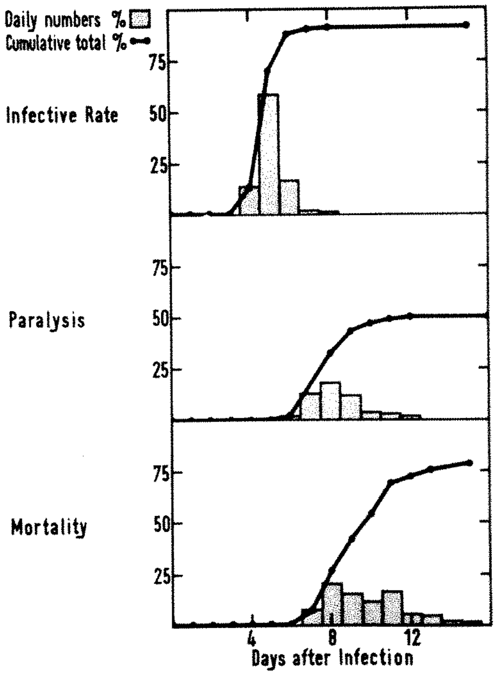

FIG. 2.—The incidence of lesions, paralysis, and the mortality, expressed as a percentage of the total.

(3) *Histological Changes*

The appearances of normal mouse skin can be seen in Fig. 3. There is a layer of muscle tissue under the subcutis, over which the relatively thick dermis, with many hair bulbs, can be seen. The epidermis is only a few cells thick. By

TABLE I.—*A Typical Experiment Showing the Daily Counts of Infected, Paralysed, Dead and Recovering Animals*

Sixty-four mice inoculated

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Infected*	—	—	—	10	50	56	56	52	42	29	19	15	13	12	12	11
Paralysed†	—	—	—	—	—	3	11	14	8	4	3	2	1	1	—	—
Died	—	—	—	—	—	—	4	10	13	10	4	2	1	—	1	—
Recovered	—	—	—	—	—	—	—	—	—	—	—	4	6	7	8	8

* Daily counts rise as lesions appear and fall as animals perish.
† Paralysed animals subsequently died.

24 h (Fig. 4) there is an infiltrate of mixed inflammatory cells. The histological appearances of an early discrete crusted lesion can be seen in Fig. 5, in which there is a dense mixed dermal inflammatory cell infiltrate, with a slight preponderance of polymorphonuclear leucocytes, and there is partial epidermal destruction. Once lesions are confluent (Fig. 6) there is a dense, but mainly lymphocytic, dermal infiltrate, with necrosis of the outer dermis and complete epidermal destruction. The surviving epidermis, in relation to lesions, may show increased mitosis (Fig. 6) or giant cell formation, disorganisation and early vesication, details of which are shown at a higher magnification in Fig. 7. The histology of an almost healed lesion (Fig. 8) shows necrotic cutaneous elements overlying completely reconstituted epidermis that is somewhat thicker than normal.

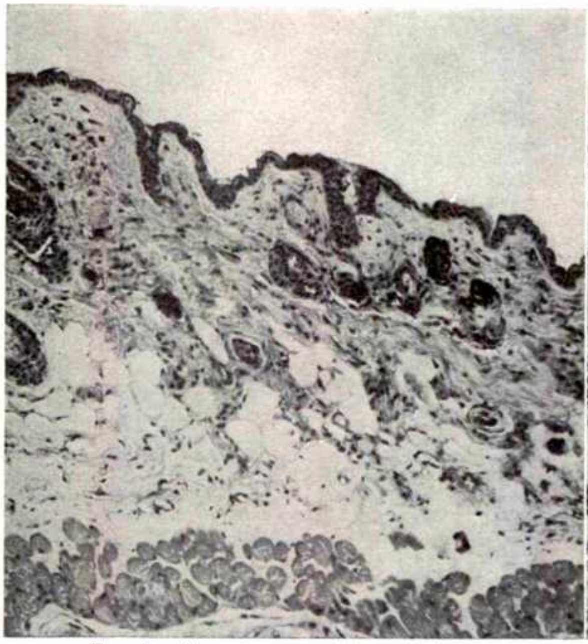

FIG. 3.—Normal mouse skin. (× 100).

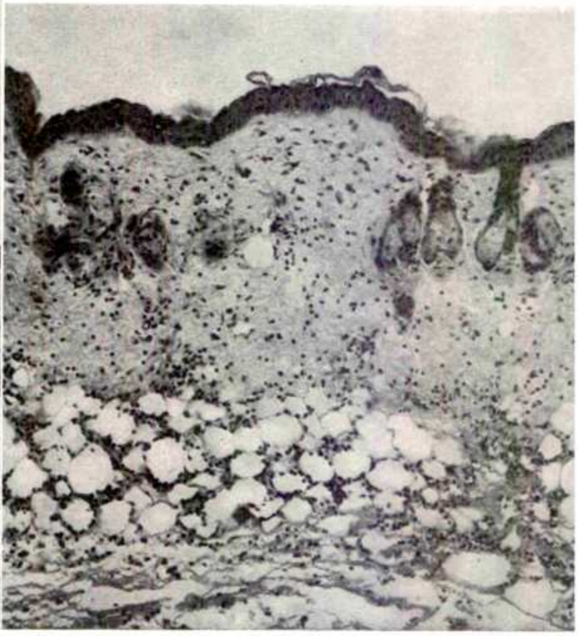

FIG. 4.—Inoculation site 24 h post-infection. (× 100)

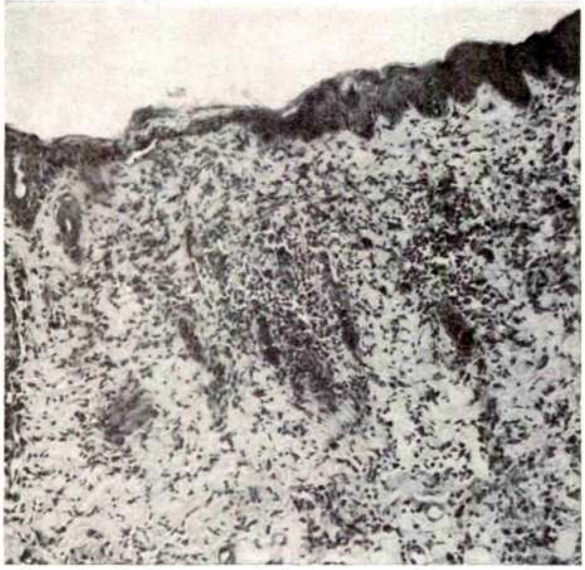

FIG. 5.—Early discrete lesion in mouse skin. (× 100).

(4) *Skin Viral Growth and Serum Neutralizing Antibody*

A rise in the HSV titre was detectable in the skin by the third day after

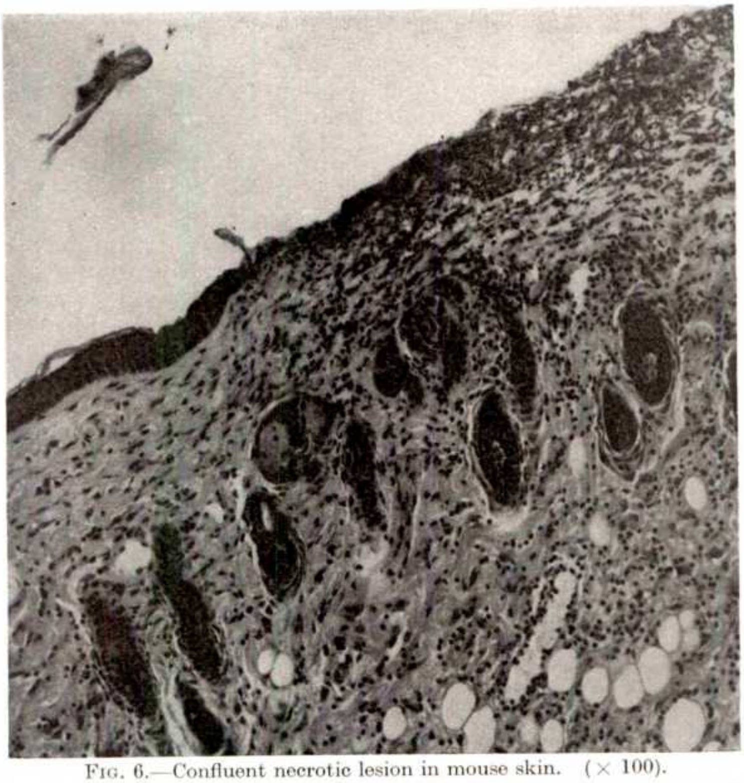

FIG. 6.—Confluent necrotic lesion in mouse skin. (× 100).

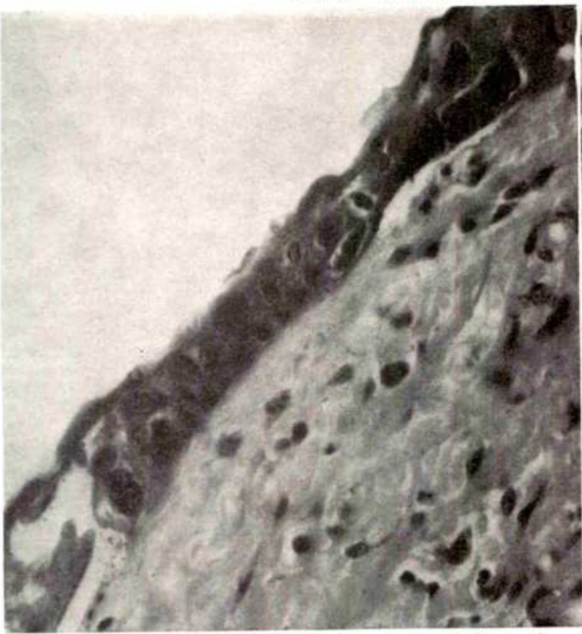

FIG. 7.—Mouse epidermis showing giant cell formation and early vesication. (× 400).

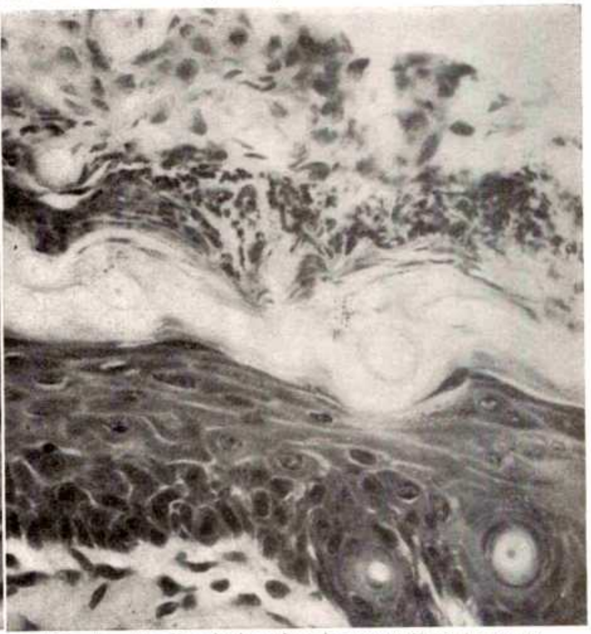

FIG. 8.—Healing lesion showing necrotic cutaneous elements overlying reconstituted epidermis. (× 400).

TABLE II.—*Virus Titration in Mice*

Experiments	Virus concentration		Mice developing lesions					Dilution infecting		50% mouse infective dose	
	TCD 50/ml	TCD 50/ inoculum	Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}	No mice	50% mice	TCD 50/ inoculum	TCD 50/ ml
1	$10^{6\cdot2}$	$10^{4\cdot9}$	13/15	7/11	4/10	1/10	0/10	10^{-4}	$10^{-1\cdot86}$	10^{3}	$10^{4\cdot3}$
2	$10^{5\cdot2}$	$10^{3\cdot9}$	11/11	9/11	3/10	1/10	0/10	10^{-3}	$10^{-1\cdot58}$	$10^{2\cdot3}$	$10^{3\cdot6}$

inoculation (Fig. 9), which reached a maximum by the fourth day and slowly fell over the next 5 days. Serum neutralizing antibody was first detected 5 days after inoculation, rising steadily over the next 10 days.

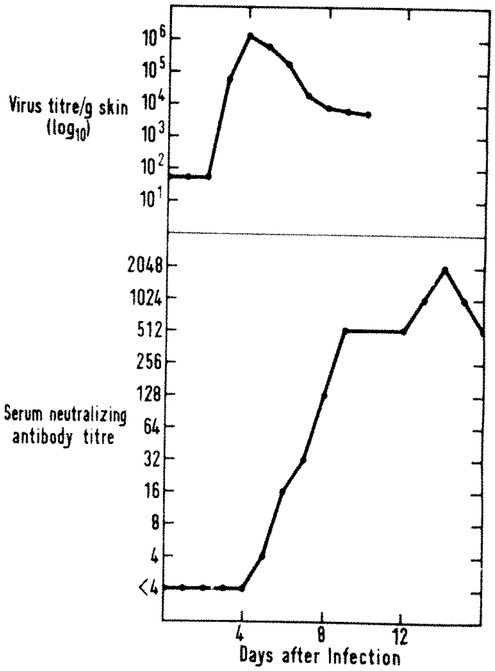

FIG. 9.—Skin viral growth and serum neutralizing antibody formation in white mice.

In Table II the results of 2 experiments are recorded in which 10-fold dilutions of 2 batches of stock virus were inoculated into groups of animals. In the first experiment the stock virus titre was 1 log higher; and, although fewer animals developed lesions at the lowest dilution, more animals were infected at higher dilutions. In addition, the dilution that failed to produce lesions, and the dilution and viral titre that infected 50% of animals, have been calculated and recorded for each experiment.

HSV was also grown from brain homogenates obtained from a small number of animals sacrificed after the onset of paralysis.

DISCUSSION

The primary aim in these experiments was the production of an animal model system in which a high incidence of cutaneous HSV infection could be consistently reproduced. An attempt was therefore made to reproduce the system described by Sydiskis and Schultz (1965), but it was unreliable unless a particular strain of Swiss white mice and a high dose of a particular strain of HSV isolated at University College Hospital Medical School were employed.

Preliminary studies established that Webster Schneider mice developed a high incidence of infection that could be accurately assessed throughout the course of infection, as non-specific skin lesions were not produced by fighting. The incidence of infection was low in Porton and Tuck No. 1 strains of mice, high in Swiss TO strain, but these animals and the Porton strain were very prone to fighting; and, in view of these findings, the Webster Schneider strain was selected as most suitable. An oral strain of HSV, also isolated locally, failed to produce lesions in all these animals, as did the HFEM strain obtained from another laboratory.

The reasons for these findings remained unexplained; but the development of zoster-like lesions under certain experimental circumstances and not under others is well known but remains unexplained. Thus, in guinea-pigs, the prior application of tar was required (Teague and Goodpasture, 1923) and Swiss mice or rats older than 9 days failed to develop similar lesions (Tanaka and Southam, 1965). The skin lesions appeared at the same time as those described by Constantine *et al.* (1965), but these workers noted the early development of vesicular lesions. Vesicles were only rarely encountered in this model, and were never noted by Constantine *et al.* (1971); but it is of interest that vesicles have been reported in all the zosteriform lesions produced in animals in other studies (Teague and Goodpasture, 1923; Tanaka and Southam, 1965; Underwood, 1968). Like Constantine *et al.* (1971), no obvious explanation of this emerged in the studies.

The incidence of infection ranged from 75 to 90%, providing that the viral titre was at least 10^{5-6}TCD$_{50}$/ml, and this was a much higher infective rate than described by Constantine *et al.* (1971) in hairless mice (36%); but these workers inoculated smaller amounts of HSV (50 TCD$_{50}$ in most experiments, and inocula greater than 200 TCD$_{50}$ were not employed). Sydiskis and Schultz used inocula containing from $10^{3\cdot3}$ to $10^{6\cdot0}$ TCD$_{50}$ of virus, and at the higher titres obtained a comparable infective rate.

As reported by other workers, all mice developing paralysis died, but a few animals with lesions died in the absence of paralysis, and in some others without paralysis the skin lesions healed and the animals appeared to recover completely. The mortality was between 70% and 80%, which was higher than that described by Sydiskis and Schultz (61%); but this was probably a reflection of the higher dose of virus and somewhat younger mice used in the study reported here.

In the histological studies, the inoculation site 24 h after infection, early discrete crusted lesions, confluent necrotic lesions and healing lesions were investigated. This was considered to be more meaningful than histological descriptions based on the number of days after infection, as adopted by Sydiskis and Schultz (1965), as lesions did not always develop on the same day or progress at a comparable rate. Twenty-four hours after inoculation there was a mixed inflammatory infiltrate in the deep dermis and subcutis; and, as the lesions developed, the infiltrate became more dense, and involved all of the dermis, which corroborates the findings of Sydiskis and Schultz (1965) and Constantine *et al.* (1971), but there were observations that were either not noted or not in agreement with these workers.

A definite preponderance of polymorphonuclear leucocytes was encountered in the early discrete lesions, but round cells predominated once the lesion had become confluent.

In a previously described mouse lung model system, in which an RNA virus (Sendai parainfluenza virus) was grown in mouse lungs, the earliest lesions in the mucous membranes were associated with a mainly polymorphonuclear leucocytic infiltration, but later on there was a dense perivascular infiltrate of mononuclear cells which were interpreted as the probable origin of much of the interferon and possibly the immune responses necessary in the recovery process (Robinson *et al.*, 1968). Similar suggestions may be relevant in the present model.

In addition, the earliest histological appearances consisted of microvesication and giant cell formation, and virtually normal epidermis at the edge of lesions showed an increased mitotic rate. The vesication and giant cell formation confirmed the previous work, suggesting that HSV replicated in the epidermis

(Sydiskis and Schultz, 1965; Tomlinson and MacCallum, 1968), and this replication may be partially responsible for the epidermal destruction and indicate that epidermal necrosis is not merely a consequence of severe dermal damage. The increased mitotic rate in the intact epidermis close to lesions was interpreted as early evidence of healing. Lastly, epidermal and superficial dermal necrosis apparently occurred at the same time.

The skin viral growth curves and serum neutralizing antibody were comparable to the findings of Sydiskis and Schultz (1965), and the titre of virus infecting 50% of mice was shown to be in the region of 10^4 TCD_{50}/ml in these studies, as compared to $10^{4.4}$ TCD_{50}/ml described by the same workers. In addition, HSV was recoverable from the brains of mice showing advancing paralysis, which confirmed that spread of HSV from the skin to the central nervous system was responsible for the paralysis and death, although no direct evidence as to the method of spread was obtained in these studies; but this has been reported elsewhere (Johnson, 1964; Constantine *et al.*, 1971).

We decided to develop the Sydiskis and Schultz primary skin infection in mice, as these animals are cheaper and easier to maintain than guinea-pigs or rabbits, particularly as large numbers of animals would be required for the planned studies. In addition, it was felt that a simple model, in which HSV injected intradermally into the skin produced a high incidence of infection and mortality, would be most appropriate to test the effect of antiherpes drugs. The model system here described has been shown to be of value in assessing the effect of specific HSV antiviral compounds, and the introduction of immunosuppressant agents early in the infective process has been shown to influence the course of the infection. Current studies along these lines will form the basis of further communications.

The authors are indebted to Professor G. Belyavin, Department of Bacteriology, and to Dr A. Jarrett, Department of Dermatological Histology, University College Hospital Medical School, for laboratory facilities. T. W. E. Robinson received personal support from The Wellcome Trust, and a grant from the Medical Research Council for technical assistance and expenses, both of which are gratefully acknowledged.

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THE CONTROL OF GLYCOGEN UTILIZATION IN SKIN; PHOSPHORYLASE *B* KINASE AND PHOSPHORYLASE *A* PHOSPHATASE

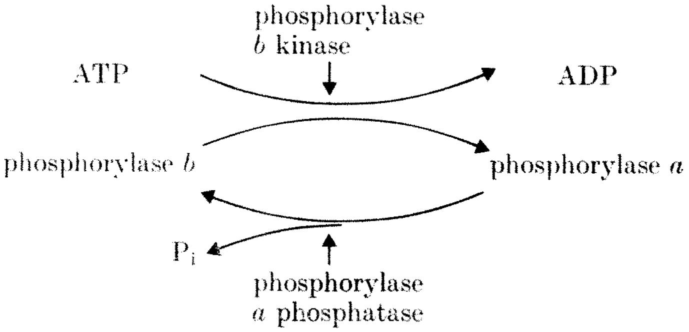
P. D. MIER AND A. H. M. SUTORIUS

SUMMARY.—The activation of phosphorylase *b* and the inactivation of phosphorylase *a* by skin homogenates have both been demonstrated for the first time. Certain properties of the enzymes catalysing these reactions (phosphorylase *b* kinase and phosphorylase *a* phosphatase, respectively) were investigated; it was concluded that the control system in skin is similar to that of muscle and liver. The possibility of certain dermatoses being associated with defects in this system is discussed.

UTILIZATION of glycogen proceeds via the reaction:

$$(\text{Glucose})_n + P_i \rightleftharpoons (\text{Glucose})_{n-1} + \text{G–1–P}$$

which is catalysed by the enzyme phosphorylase. This enzyme exists in an inactive form (phosphorylase *b*) and an active form (phosphorylase *a*). Control of the rate of glycogen utilization by the cell is achieved by the interconversion of these 2 forms:



The enzymes responsible for the interconversion (phosphorylase *b* kinase and phosphorylase *a* phosphatase) have themselves both been shown to possess active and inactive forms. In addition, various "small" molecules (including cyclic AMP) have been reported to participate at different points in these equilibria. The function of the system as a whole seems to be two-fold: the cell can maintain an internal homeostasis with respect to metabolites such as glucose and phosphate ions; yet, at the same time, it is able to respond with alacrity to extracellular stimulation by certain hormones. The general features of glycogen control in

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muscle and liver have now been clarified; the subject is reviewed by Fischer *et al.* (1970).

There is considerable evidence that glycogen plays an active role in the intermediary metabolism of skin. It is found in substantial quantity whenever an unusually high level of metabolic activity is present, for example in foetal epidermis, in the sweat glands and external root sheaths of adult skin, and in many pathological conditions. Adachi (1961) showed that there is a remarkably rapid turnover of cutaneous glycogen, and Halprin and Ohkawara (1966) measured the activity of certain enzymes associated with cutaneous glycogen metabolism. Surprisingly, however, nothing whatsoever is known about the control mechanism in skin beyond a single report that both *a* and *b* forms of phosphorylase can be demonstrated (Mier and Cotton, 1967). We have therefore investigated the phosphorylase *b* kinase activity and the phosphorylase *a* phosphatase activity of skin homogenates; our results are reported below.

MATERIAL AND METHODS

Mouse skin was prepared and homogenized as described in a previous report (Mier and Cotton, 1966). In general, reagents were of analytic grade; the enzymes were purchased as crystalline suspensions from the Sigma Chemical Co. The phosphorylase *b* used as substrate had a specific activity of 1,250 units/mg, and preliminary assays showed it to contain about 6% phosphorylase *a*. The phosphorylase *a* had a specific activity of 1,680 units/mg; no significant contamination with the *b* form was found.

Measurement of phosphorylase a *and* b *activity.*—This was carried out fluorimetrically; glucose-1-phosphate was converted to glucose-6-phosphate with phosphoglucomutase, and the latter measured by the NADP coupled oxidation in the presence of glucose-6-phosphate dehydrogenase. The instrument used was the " Fluorispec " (Baird Atomic) with an external recorder. Measurements were carried out at room temperature.

The incubation mixture contained orthophosphate (320 μmole, pH 6·7), EDTA (1 μmol), $MgCl_2$ (6 μmol), NADP (0·2 μmol), glycogen (10 mg), PGM (20 μg) and G-6-P dehydrogenase (4 μg) in a total volume of 1·5 ml. The reaction was initiated by the addition of 0·5 ml of the phosphorylase sample (usually containing 1–10 μg total phosphorylase), and the reaction followed by the increase in fluorescence at 460 nm (excitation 350 nm). When the plot had been linear for about 10 min, 0·1 ml 5′-AMP was added to the cuvette, and the reaction allowed to proceed for a further 10 min. Phosphorylase *a* activity was derived from the slope of the first plot, and total phosphorylase activity from the slope of the second. Preliminary experiments verified that the slopes were proportional to added phosphorylase within the range of concentrations used.

Assay of phosphorylase b *kinase.*—The following were mixed and brought to 37 °C: 0·2 ml of phosphorylase *b* (500 μg/ml) in 0·2 mol/l tris buffer (pH 8·6), 0·05 ml of a fresh solution of 0·15 mol/l cysteine hydrochloride (neutralized with NaOH) and 0·05 ml of a solution containing 0·1 mol/l $MgCl_2$ and 0·03 mol/l ATP. The reaction was initiated by the addition of 0·2 ml skin homogenate (usually 50 mg/ml) in 0·2 mol/l tris buffer (pH 8·6). After an appropriate interval (usually 10 min) the reaction was terminated by pipetting an aliquot of 0·1 ml of the incubation mixture into 3·9 ml of ice-cold phosphate buffer (0·04 mol/l, pH 6·7) containing 0·015 mol/l cysteine. This was centrifuged briefly, and phosphorylase *a* and *b* activities in the supernatant were measured as described above.

Assay of phosphorylase a *phosphatase.*—0·5 ml of a solution of phosphorylase *a* (200 μg/ml) in 0·2 mol/l tris buffer (pH 7·4) was brought to 37 °C. The reaction was initiated by the addition of 0·5 ml of a skin homogenate (usually 50 mg/ml) in 0·2 mol/l tris buffer (pH 7·4). After the reaction period (usually 20 min), the reaction was terminated and phosphorylase *a* and *b* measured as before.

Progress-curves.—Incubation mixtures were set up for the assay of phosphorylase *b*

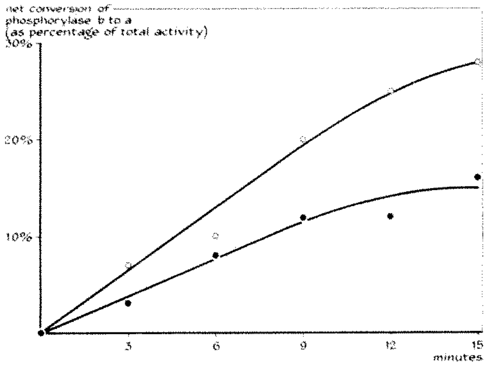

FIG. 1.—Progress-curves for the phosphorylase *b* kinase reaction. Conditions were as described in the text.

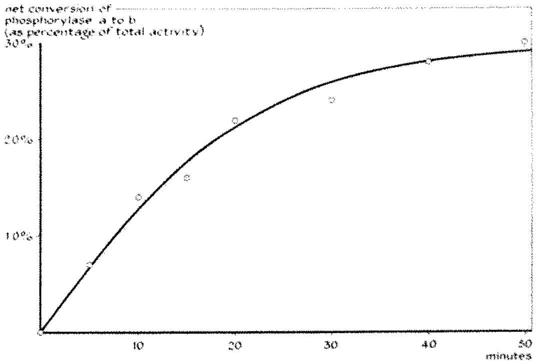

FIG. 2.—Progress-curve for the phosphorylase *a* phosphatase reaction. Conditions were as described in the text.

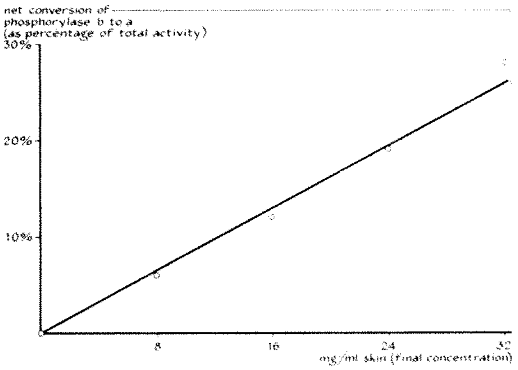

FIG. 3.—Conversion of phosphorylase *b* to *a* as a function of quantity of added homogenate. All incubations were for 10 min.

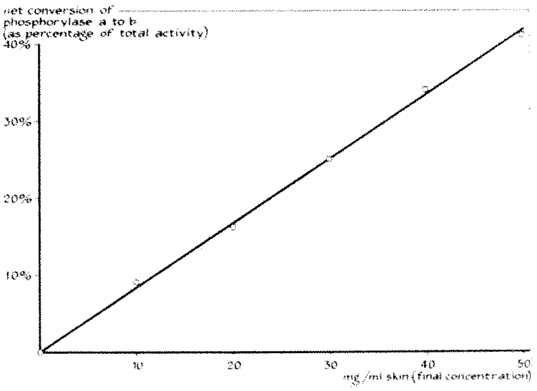

FIG. 4.—Conversion of phosphorylase *a* to *b* as a function of quantity of added homogenate. Incubations were for 20 min.

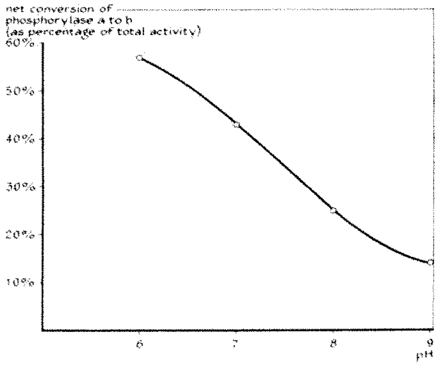

FIG. 5.—Variation of phosphorylase *a* phosphatase activity with pH.

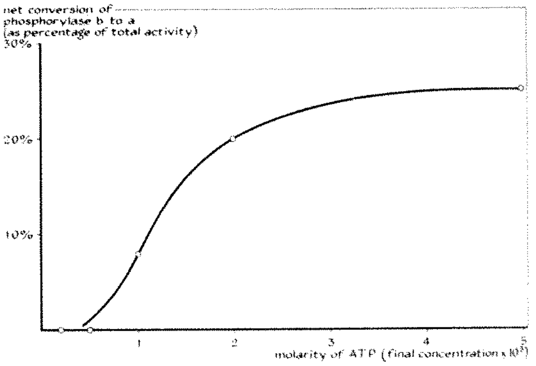

FIG. 6.—Phosphorylase *b* kinase activity as a function of ATP concentration.

kinase and phosphorylase *a* phosphatase exactly as described above, and samples were withdrawn at intervals for the measurement of phosphorylase *a* and *b*. Control experiments were carried out, omitting the skin homogenate.

Variation with quantity of skin.—A series of experiments was carried out in which phosphorylase *b* kinase and phosphorylase *a* phosphatase activities were measured using dilutions of a skin homogenate in the range 20–100 mg/ml. The incubation period was 10 min for the kinase and 20 min for the phosphatase.

Variation with pH.—This was investigated in the case of both enzymes, using tris buffers in the range pH 6–9. In these assays the skin homogenate was prepared in distilled water.

ATP-dependence of phosphorylase b *kinase.*—An attempt was made to measure the K_m of the kinase for ATP. A series of assays was conducted in which the ATP concentration was varied over the range 5×10^{-3} mol/l to 2×10^{-4} mol/l; in all other respects the assay was as described previously.

RESULTS

Progress-curves for the phosphorylase *b* kinase reaction are shown in Fig. 1; the results of 2 different experiments are illustrated. A progress-curve for the phosphorylase *a* phosphatase reaction is shown in Fig. 2. It is seen that, for both enzymes, the reaction was linear for only a short period. Using a fixed incubation period, however, the reaction rates were proportional to the quantity of added homogenate over the whole of the ranges studied (Fig. 3, 4). No activity was found in the control experiments in which the skin homogenate was omitted.

The kinase showed very little variation with pH over the range pH 7–9; the activity of the phosphatase, however, decreased with increasing pH over the physiological range (Fig. 5). The effect of ATP concentration on the reaction velocity of the kinase reaction is shown in Fig. 6; a Lineweaver-Burke plot derived from these values is non-linear (indicating loss of ATP via competing enzyme systems), but suggests an upper limit for the K_m of about 10^{-3} mol/l.

DISCUSSION

The continuous fluorimetric assay of phosphorylase employed in this investigation was found to be a considerable improvement on the more usual approach, which is to use a stop-time assay and measure the inorganic phosphate released in the " back " reaction. In particular, the routine addition of 5′-AMP to each sample (providing a measure of the total phosphorylase activity) enabled the proportion of phosphorylase *a* to be calculated directly as a ratio between the two slopes; this avoided any errors resulting from instrumental variations.

Our results demonstrate beyond doubt that there is a rapid enzymatic interconversion of phosphorylase *a* and *b* in skin. Clearly it is difficult to obtain reliable quantitative information regarding such a complex system by the use of crude tissue homogenates; nevertheless, the evidence presented in this report suggests that both of the enzymes studied are similar to their counterparts in liver and muscle. The upper limit of 10^{-3} mol/l for the K_m of phosphorylase kinase with respect to ATP, for example, compares reasonably with the value of $3{\cdot}1 \times 10^{-4}$ mol/l reported by Krebs *et al.* (1964) for a purified preparation from rabbit muscle.

Thus it seems very probable that the control mechanism for the activation of phosphorylase in skin is similar to that in the better-known tissues. This knowledge may perhaps offer us new territory to explore in our search for the molecular basis of certain diseases. In atopy, for example, an abnormality has been postulated in the response of the cell to catecholamines (Szentivanyi, 1968); the enzyme catalysing the first step in this process has been shown to function normally (Mier and Urselmann, 1970), but the subsequent activation of phosphorylase *b* kinase by cyclic-AMP seems to be an alternative possibility. Conversely, in a disease such as psoriasis, which is characterized by an overactive epidermal metabolism, it seems rational to look for a defect in the phosphorylase *a* phosphatase system. Clearly this might well be a rewarding area for further research.

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TREATMENT OF VITILIGO WITH ORAL 4,5′,8-TRIMETHYLPSORALEN (TRISORALEN)

S. S. BLEEHEN*

SUMMARY.—Thirty-two patients with vitiligo were treated with oral 4,5′,8-trimethylpsoralen (TMP) and subsequently exposed to various sources of long wave u.v. light, including natural sunlight. The patients were treated once or twice weekly over a period of 4–28 months, taking 15–20 mg of TMP at least 2 h before exposure to u.v. light. The amount of exposure to u.v. light was carefully graduated, and slowly increased according to the patient's tolerance.

Six patients showed considerable repigmentation of the areas of vitiligo; 9 others had a significant cosmetic improvement. Areas of vitiligo on the face, neck and trunk were more responsive to treatment than those on the distal parts of the limbs. Natural sunlight in the summer months appears to be much more effective in inducing repigmentation of vitiliginous skin than artificial sources of long wave u.v. light. No adverse effects due to the drug were noted.

It is suggested that treatment with systemic TMP is effective and safe and is of use in selected cases of vitiligo.

DURING the early 1950's meladinine (Lerner *et al.*, 1953), and later 8-methoxypsoralen alone (Kanof, 1955), were found to be of value in inducing repigmentation of vitiliginous skin when applied topically or taken by mouth. In Britain, meladinine has been the main preparation used for the treatment of selected cases of vitiligo, and has been responsible for a widely held clinical impression that psoralens are not very effective. There has also been some concern about possible hepatotoxicity.

Since 1964, a new synthetic furocoumarin derivative, 4,5′,8-trimethylpsoralen (Trisoralen), has been used in the United States and elsewhere for the treatment of vitiligo. This compound is more potent than 8-methoxypsoralen in its photosensitizing effect (Pathak and Fitzpatrick, 1959: Pathak *et al.*, 1967), and the results of therapy suggest that it may be more effective as a therapeutic agent (Hamada, 1966; Fitzpatrick *et al.*, 1966). No adverse effects due to systemic administration of this drug have so far been reported, despite its widespread use.

The purpose of this paper is to report 32 patients with vitiligo who have been treated with systemic trimethylpsoralen over a period of 3 years. The duration of treatment has varied from a minimum of 4 months to as long as 28 months. The

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clinical results indicate that oral administration of trimethylpsoralen and subsequent exposure to long wave u.v. light are of help to a significant number of patients.

PATIENTS AND METHODS

Over the past 3 years, 32 patients with vitiligo (18 females and 14 males) were selected for treatment with systemic trimethylpsoralen. These patients are summarized in Table I, and were selected out of 120 patients referred. The indication for treatment was a marked cosmetic disability. The age range of the patients was 10–60 years and the mean age 32 years. Twelve patients were coloured and of various racial origins; the others were British and white. A number of patients had other diseases, as shown in Table II; some of these disorders are well recognized as occurring in association with vitiligo. None of the patients had any previous hepatic disease, and all were in good

TABLE I.—*Patients with Vitiligo Treated with Trimethylpsoralen*

Case No.	Sex and age	Racial colour	Duration of disease	Distribution of lesions	Length of treatment (months)	Response: % overall repigmentation
1	M 19	Coloured	3 years	Face, trunk, limbs	28	75
2	F 46	White	4 years	Face, trunk, limbs	20	10–20
3	F 42	White	11 years	Face, trunk, limbs	8	Nil
4	M 43	White	10 years	Face, trunk, limbs	4	Nil
5	F 35	White	3 years	Face, trunk, limbs	12	20
6	M 35	Coloured	18 months	Face	12	50
7	F 31	White	8 years	Face, trunk, limbs	8	< 10
8	F 40	Coloured	15 years	Face, trunk, limbs	6	< 10
9	M 28	Coloured	4 years	Face, trunk, limbs	12	20
10	F 24	Coloured	1 year	Face and arms	10	Nil
11	F 38	White	23 years	Face, trunk, limbs	22	40
12	F 31	White	11 years	Face, trunk, limbs	20	30
13	M 25	Coloured	2 months	Face and hands	10	10
14	F 14	White	6 years	Face, trunk, limbs	18	20
15	M 40	White	6 years	Face, trunk, limbs	8	20
16	F 31	White	3 years	Face, trunk, limbs	6	30
17	F 24	Coloured	20 years	Face, trunk, limbs	6	Nil
18	F 26	Coloured	15 years	Face, trunk, limbs	5	Nil
19	M 24	Coloured	19 years	Face, trunk, limbs	5	< 10
20	F 46	White	8 years	Face, trunk, limbs	4	10
21	F 10	White	2 years	Face, trunk, limbs	5	Nil
22	M 40	Coloured	1 year	Face, trunk, limbs	4	Nil
23	M 44	Coloured	30 years	Face, trunk, limbs	4	Nil
24	F 24	White	6 years	Face, trunk, limbs	4	50
25	F 60	White	14 years	Face, trunk, limbs	7	10
26	F 20	Coloured	12 years	Face, trunk, limbs	7	Nil
27	M 26	White	5 years	Face, trunk, limbs	7	Nil
28	M 35	White	10 years	Face, trunk, limbs	10	10
29	M 41	White	1 year	Face, trunk, limbs	4	Nil
30	M 38	White	5 years	Face, trunk, limbs	8	10–20
31	F 15	White	10 years	R. side trunk and leg	4	Nil
32	M 35	White	20 years	Face, trunk, limbs	5	Nil

TABLE II.—*Other Conditions Present Among 32 Patients with Vitiligo*

Disease	Case No.	Total
Thyroid disease (Thyrotoxicosis)	3, 5, 7 and 25	4
Alopecia areata	7, 8 and 29	3
Diabetes mellitus	7	1
Atopic eczema and asthma	4	1
Eczema	10	1
Behcet's syndrome	22	1

health. Hb., W.B.C. and differential, liver function tests, serum transaminases and electrolytes were estimated before and during treatment. In addition, tests for anti-nuclear factor and organ-specific autoimmune antibodies were performed on serum obtained from these patients. Photographs were taken at the start of treatment and at various times during treatment under as near as possible identical conditions, both with and without u.v. illumination. The photographs were used to assess the amount of repigmentation and the response to therapy. Patients were seen at 4–6 weekly intervals, and all were treated as out-patients. Each patient was instructed to take 15 or 20 mg (3–4 tablets) of trimethylpsoralen at least 2 h before exposure to long wave u.v. light or natural sunlight. Various artificial sources of long wave u.v. light were used, but the majority of patients were treated with a bank of fluorescent light tubes (emitting 320–420 nm wavelengths). The amount of exposure to u.v. light was gradually increased over a period of several months, according to the patient's tolerance. During the summer months, the patients were instructed to take their tablets in the morning, especially if it was sunny, and to sunbathe around midday, exposing as much of the skin as possible. Frequency of treatment was no more than once every 5 days; and, if a marked erythema of the skin developed, the next treatment was delayed until this erythema subsided. The patients in these series were treated for a minimum of 4 months, and in some cases for as long as 28 months. The amount of repigmentation was assessed clinically and from objective measurements of tracings from photographs. The results of therapy were also assessed by the individual patients by cosmetic improvement, if any, and by an increased tolerance to sunlight of areas of vitiligo.

RESULTS

The results of treatment are summarized in Table III. Of the patients treated, 6 had a good response to therapy. It is significant that it was these patients who were among those treated the longest. Four of these patients were British and white, and 2 were coloured. Nine other patients had a significant amount of repigmentation of the skin, mainly on the face and neck, sites of prime cosmetic importance to the patients, who were delighted with their improvement. Although young patients appeared to respond more rapidly than older patients, the response did not seem to be related to the duration of the lesions before treatment. Case 11 had vitiligo for 23 years and had an excellent response to therapy (Fig. 1, 2, 3, 4). Areas of vitiligo on the face and neck, especially the periorbital region, responded best to treatment (Fig. 5, 6: Case 1), whilst those on the trunk and limbs responded less well. Lesions on the backs of the hands, dorsum of the feet, elbows and knees, i.e. sites over bony prominences that are often subject to friction and trauma, were more resistant to treatment. Areas of vitiligo in which the hairs were also depigmented did not respond to treatment, nor did depigmented hairless areas, such as the lips (Fig. 5, 6) and genitals.

TABLE III—*Summary of Results of Treatment with Trimethlylpsoralen*

Clinical response	No. of cases	% of total
Good	6	19
Fair	9	28
Poor or nil	17	53

The rate of repigmentation was very variable. The first indication of a response was usually the appearance of perifollicular islands of pigment that gradually enlarged to coalesce and form larger patches of pigment. There was also a gradual spread of pigment from round the edge of the lesion into the area of vitiligo.

In none of the patients was a total repigmentation of all the areas of vitiligo observed, although in 6 cases the total amount of repigmentation of the areas of vitiligo was between 30% and 75%. Treatment has been discontinued for a number of months in several patients without producing a relapse of their vitiligo. Only 1 patient developed a number of new depigmented areas of skin while on treatment. This patient, Case 10, also had eczema, and these lesions developed in the eczematous areas.

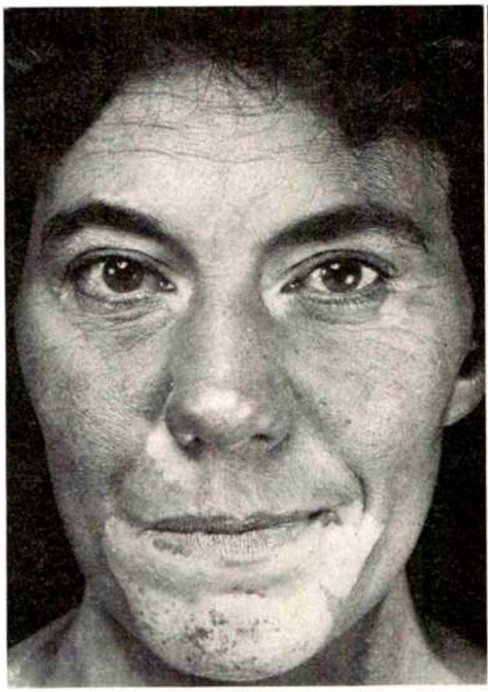

FIG. 1 (Case 11).—Vitiligo on face. Few perifollicular islands of repigmentation on chin after 6 months of therapy, coinciding with advent of sunny weather.

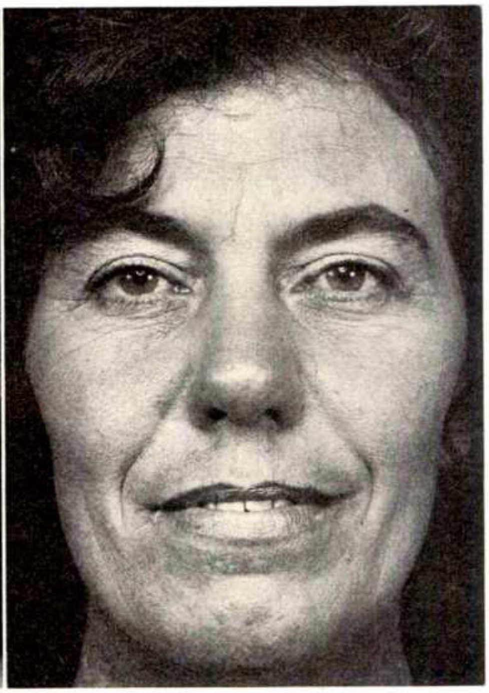

FIG. 2 (Case 11).—Almost complete repigmentation of areas of vitiligo on face, after 22 months of treatment.

Natural sunlight was more effective than artificial sources of long wave u.v. light. Often the first response to treatment occurred with the advent of sunny weather in May to August.

Apart from the cosmetic improvement with therapy, 6 patients commented that they were able to tolerate a greater amount of sun exposure in their areas of vitiligo as a result of treatment with systemic trimethylpsoralen. None of the patients developed blisters of the skin. In contrast, topical therapy with meladinine or with trimethylpsoralen, followed by over-exposure to long wave u.v. light, not infrequently produces blistering of the skin where these psoralens have been applied. Although it was observed that the majority of patients treated with systemic trimethylpsoralen developed a delayed erythema of the exposed areas of vitiligo, especially during the summer months, only in a few was this erythema persistent or severe enough to give rise to symptoms. Treatment

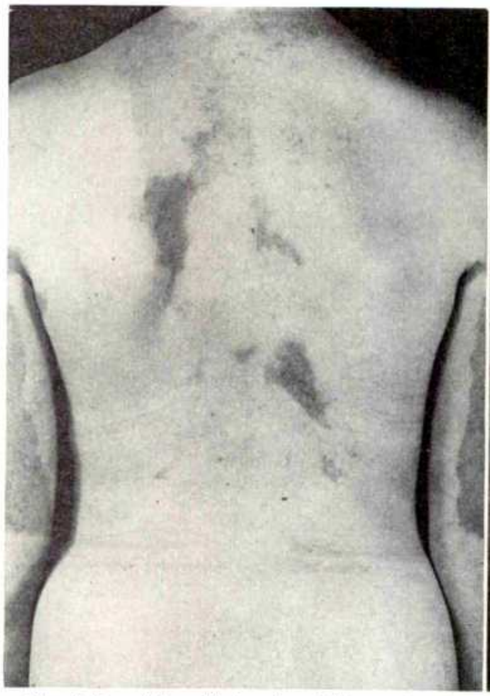

FIG. 3 (Case 11).—Extensive vitiligo on trunk before start of treatment.

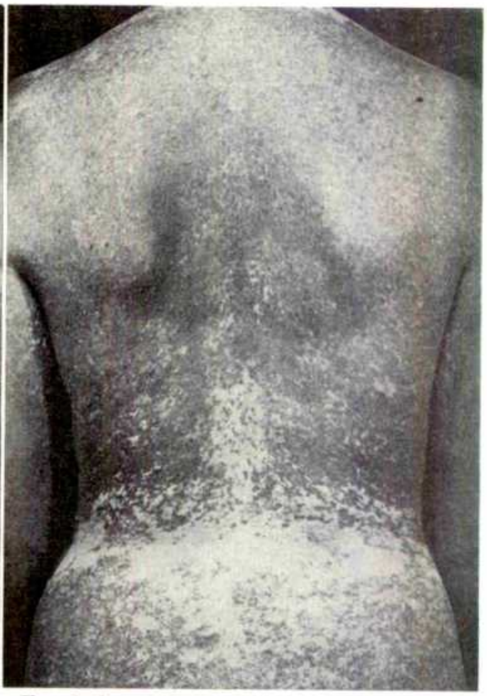

FIG. 4 (Case 11).—Considerable repigmentation after 22 months of therapy.

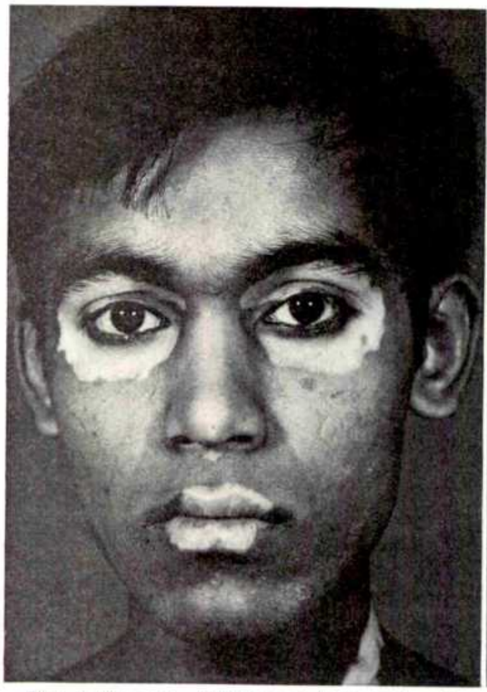

FIG. 5 (Case 1).—Vitiligo on face and neck (before treatment).

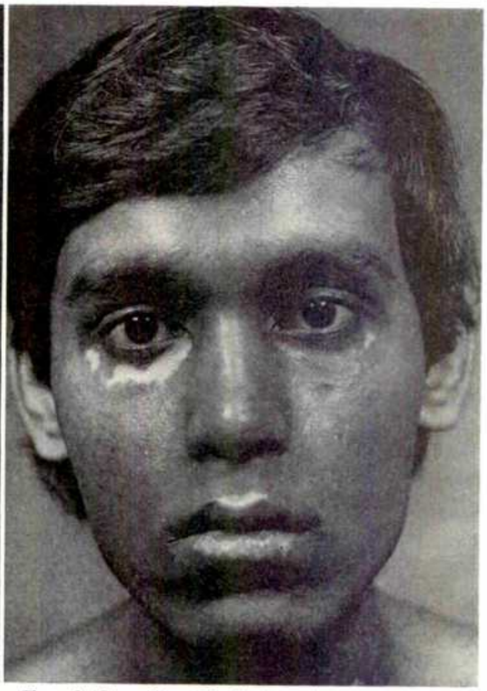

FIG. 6 (Case 1).—Marked repigmentation of areas of vitiligo on face and neck (28 months of treatment). Only slight repigmentation of lips.

of 2 patients had to be discontinued for several weeks because of itchy erythema and scaling of the treated areas. In 1 patient, Case 4, treatment was discontinued after 4 months because of nausea and occasional diarrhoea soon after taking the tablets. However, this patient had experienced similar symptoms with other unrelated drugs. Serial tests of liver function were all within the normal range. One patient, Case 7, who was not overtly diabetic before treatment, developed glycosuria while on therapy with systemic trimethylpsoralen. This was considered to be coincidental, and this patient also developed alopecia areata.

DISCUSSION

The mode of action of psoralens in inducing repigmentation of the skin is as yet unknown. The observation that repigmentation of skin is often perifollicular led to the suggestion that treatment with psoralens might induce colonization of depigmented skin by melanocytes originating from pigmented hair bulbs (Pegum, 1955). However, areas of vitiligo occurring in the hairless skin of the genitalia and lips may also become repigmented, indicating that the movement of functional hair melanocytes to areas of vitiligo is not the sole method of repigmentation (Fitzpatrick, 1959). A possible explanation of the mode of action is that the drug activates the few remaining functional and dopa-positive melanocytes which are present in areas of so-called " relative vitiligo " (Jarrett and Szabo, 1956). More recently, Clark *et al.* (1968) have shown that psoralens evoke a specific mitotic effect on melanocytes. Similarly, autoradiographic studies (Africk and Fulton, 1971) have shown that, in repigmenting vitiligo lesions treated with topical trimethylpsoralen, more than 1 % of dopa-positive melanocytes incorporated tritiated thymidine label in their nuclei, indicating impending mitosis. Psoralens are also known to undergo covalent linkage with DNA upon irradiation (Pathak and Kramer, 1969).

Nearly 50 % of the patients treated in this trial showed recovery of pigment in a significant proportion of the total area of vitiligo. The areas which responded most readily to treatment were fortunately the exposed areas of the face and neck, and many patients who showed minimal response were well pleased with the cosmetic result.

Despite the widespread use of trimethylpsoralen in the United States, Japan and elsewhere (Fitzpatrick, 1970; Hamada, 1966; Fitzpatrick *et al.*, 1966; Sato, 1967), there have been no reports of adverse toxic effects. In Britain there has been only 1 report of the use of oral trimethylpsoralen in the therapy of vitiligo (Dawber, 1970). In this limited and short-term study, trimethylpsoralen produced a cosmetic improvement in 2 of 7 patients treated. None of the patients had any side effects.

While treatment with systemic trimethylpsoralen is not advocated for all patients with vitiligo, it appears to be safe and effective and is of value in selected cases where there is considerable cosmetic disability and resultant mental distress.

I wish to thank all the dermatologists who have referred these patients to me, in particular colleagues at St John's Hospital for Diseases of the Skin and at University College Hospital; and Professor T. B. Fitzpatrick and Dr M. A. Pathak of Harvard Medical School for their helpful advice. I wish to thank also the physiotherapists at these hospitals and at several others for their assistance and cooperation in providing u.v. treatment. Trimethylpsoralen (Trisoralen) tablets were supplied by Paul B. Elder and Company, U.S.A. This drug is not available in the U.K.

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THE EFFECT OF PROLONGED TOPICAL APPLICATION OF AN ANTICHOLINERGIC AGENT ON THE SEBACEOUS GLANDS

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SUMMARY.—The application of a topical anticholinergic agent (poldine methylmethosulphate) to the forehead skin of 10 patients with mild acne vulgaris twice daily for 4 weeks produced a small but significant reduction in sebum excretion. This is compatible with the suggestion that the control of sebum secretion may have a cholinergic component, but the effect is small.

THERE is a plexus of nerve fibrils around the sebaceous glands (Nakanishi, 1930; Boeke, 1934; John, 1942; Champy *et al.*, 1945; and others), but there is little evidence that these nerves affect the function of the glands. Montagna and Ellis (1957) showed that these nerve fibrils are rich in cholinesterase, and we have therefore studied the effect of prolonged application of a topical anticholinergic agent on sebaceous gland function.

METHOD

We studied 4 male and 6 female patients with mild acne vulgaris, whose ages ranged from 17 to 24 years. The sebum excretion rate (S.E.R.) was measured on each side of the forehead in each patient by the method of Strauss and Pochi (1961), as modified by Cunliffe and Shuster (1969), and the measurements were repeated a week later. The next week a further measurement was made, starting 60 min after the application of a 5% ethanolic solution of poldine methylmethosulphate (Nacton) to both sides of the forehead. Each patient continued to apply this solution to the forehead twice daily for the next 4 weeks, after which a final measurement of sebum excretion rate was made. No untreated control subjects were studied over the same period, but the effect of possible environmental changes was excluded to some extent by staggering the observations over a period of 3 months so that the control and treatment periods overlapped. The efficacy of the anticholinergic preparation was checked at the end of the experiment in each patient by an iodine-starch method to confirm that forehead sweating had been inhibited.

RESULTS

The results are shown in Table I. Each S.E.R. is the mean of the values from the right and left sides of the forehead.

The mean sebum excretion rates during the control period were $1{\cdot}43 \pm 0{\cdot}17$ and $1{\cdot}42 \pm 0{\cdot}17$ $\mu g/cm^2/min$ respectively. A single application of poldine methylmethosulphate produced no change in mean sebum excretion rate ($1{\cdot}42 \pm 0{\cdot}18$ $\mu g/cm^2/min$), but after twice daily applications to the forehead for 4 weeks, the mean sebum excretion rate fell to $1{\cdot}28 \pm 0{\cdot}15$ $\mu g/cm^{-2}/min$. Taking

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the group as a whole the mean percentage decrease in S.E.R. after prolonged application was 8·6 ± 3·9%. This decrease just failed to reach the level of statistical significance ($0{\cdot}1 > P > 0{\cdot}05$). When, however, the difference was analysed for each individual, the difference was found to be significant. This is indicated in Table II, which shows the mean of the 2 control estimations of S.E.R. for each patient, and the differences after a single application and after 4 weeks application of poldine. The mean difference following 4 weeks treatment with poldine (0·14 ± 0·05) was significantly different from zero ($P < 0{\cdot}02$).

TABLE I.—*The Effect of Topical Application of 5% Poldine Methylmethosulphate on the Sebum Excretion Rate*

	Sebum excretion rates (μg/cm²/min)			
Subject	Control 1	Control 2	Following a single application of poldine	Following 4 weeks of b.d. applications of poldine
1	1·30	1·18	1·24	1·17
2	1·51	1·48	1·27	1·08
3	1·57	1·74	1·56	1·35
4	0·78	0·69	0·75	0·81
5	1·79	1·91	1·97	1·62
6	0·85	0·99	0·77	0·78
7	1·11	0·91	0·98	1·13
8	1·92	1·86	1·95	1·63
9	0·97	1·10	—	0·91
10	2·47	2·38	2·26	2·32
Mean	1·43	1·42	1·42	1·28
n	10	10	9	10
Var.	0·29	0·29	0·30	0·22
S.D.	0·53	0·54	0·55	0·47
S.E.M.	0·17	0·17	0·18	0·15

TABLE II

	Sebum excretion rates (μg/cm²/min)		
Subject	Mean of 2 control measurements	Mean control S.E.R. *minus* S.E.R. after a single application of poldine	Mean control S.E.R. *minus* S.E.R. after 4 weeks' application of poldine
1	1·24	0	+0·07
2	1·49	+0·22	+0·41
3	1·65	+0·09	+0·30
4	0·73	−0·02	−0·08
5	1·85	−0·12	+0·23
6	0·92	+0·15	+0·14
7	1·01	+0·03	−0·12
8	1·89	−0·06	+0·26
9	1·03	—	+0·12
10	2·42	+0·16	+0·10
Mean	1·42	+0·05	+0·14
n	10	9	10
Var.	0·28	0·01	0·03
S.D.	0·53	0·11	0·16
S.E.M.	0·17	0·04	0·05

DISCUSSION

Following the prolonged application of a topical anticholinergic agent we found a small but significant decrease in sebum excretion rate. This may have been due to a blockade of cholinergic nerve impulses to the sebaceous gland, or it may have been secondary to some other effect, such as change in sweat secretion, skin temperature or even to changes in the stratum corneum. No decrease in S.E.R. was observed shortly after a single application of the same preparation, but this single application produced only a slight reduction in sweat secretion.

Kligman and Shelley (1958) and Ikai (1963) have claimed that sweat and sebum excretion are independent. Ikai (1959) found that the intracutaneous injection of epinephrine and acetylcholine had no effect on the S.E.R. He measured only the short-term response to a single injection, however; and, since the sebaceous glands are holocrine, with a relatively long turnover time (Epstein and Epstein, 1966), it seems likely that their response would be delayed.

Doupe and Sharp (1943) and Hodgson Jones *et al.* (1952) were unable to detect any change in sebum excretion rate in a small number of patients after denervation, but there have been many convincing case-reports of localized seborrhoea following a nerve lesion (Marschalko, 1905; Nexmand, 1944; Bettley and Marten, 1956; and others). Burton *et al.* (1971), in a recent quantitative study, found a variable effect in that some patients with a unilateral facial nerve lesion had seborrhoea on the paralysed side whilst others had a decreased excretion rate on the affected side.

Whilst our present findings support the suggestion that the control of sebum secretion may have a cholinergic component, the data suggests that this effect is very small.

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EFFECT OF TOPICAL TETRAYNOIC ACID ON SEBUM EXCRETION

T. S. HAROON, J. HALL AND A. HAROON

SUMMARY.—The topical application of 2% tetraynoic acid in ethanol +20% of propylene glycol failed to produce a significant reduction in sebum excretion in subjects with acne.

5 : 8 : 11 : 14-tetraynoic acid when given orally was found to be effective in lowering the sebaceous excretion in man (Strauss and Pochi, 1967). Trials were undertaken to evaluate its efficacy when applied topically for treatment of acne vulgaris. Shuster and Burton (1970) found a slight but significant decrease ($P > 0{\cdot}05$) in sebum excretion of 7 acne patients after 6 to 8 weeks' treatment. Haroon *et al.* (1970) noted a similar but slower response in volunteers with acne as compared with those without acne after 6 weeks treatment. After 12 weeks, however, the former showed a greater reduction in sebum excretion, but the number of volunteers was too small to provide a significant value. In both these studies tetraynoic acid was used as a 2% solution in ethanol.

The results were sufficiently promising to encourage us to plan a double blind cross-over study with a new formulation consisting of 2% tetraynoic acid in ethanol +20% of propylene glycol. The addition of propylene glycol was believed to promote an increase in the absorpton of tetraynoic acid by the skin (Holgate, 1970).

METHODS

Eight volunteers with acne and 10 without acne participated. Three abandoned the trial due to personal reasons before completion. Of the remaining 15, 6 were males and 9 females, their ages ranging between 17 and 36. Estimation of their sebaceous excretion was carried out according to the method of Strauss and Pochi (1961). The sebum excretion level in each individual before treatment was measured as the mean of 5 consecutive readings. Each person was then given an aerosol containing either the active substance or an identical-looking placebo according to a randomized code. The volunteers were asked to apply a metered dose from the aerosol to the forehead, twice daily for 6 weeks. Weekly estimation of the sebaceous excretion was carried out. After 4 weeks, to allow for any possible lag in the restoration of the function of sebaceous glands, the treatment was reversed, so that those who had received the tetraynoic acid preparation initially now received the placebo and vice-versa. Thus volunteers acted as their own controls. A further 6 readings at weekly intervals were recorded and the results compared.

RESULTS

The results in Table I, II show that there was a significantly greater excretion of sebum in acne as compared with normal volunteers before treatment, but no

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significant difference at any other stage. The sebum excretion of both acne and normal volunteers was reduced during active treatment, but not significantly. However, when both groups were taken together, the combined reduction was just significant ($P > 0{\cdot}05$). Response to placebo treatment was interesting. While the normals had an insignificant increase in the sebum excretion, the acne volunteers showed a marked reduction ($P > 0{\cdot}01$).

TABLE I.—*Sebum Excretion (mg/10 cm²/3 h)*

	Pretreatment Mean	End of active treatment Mean	End of placebo treatment Mean
All	0·709	0·536	0·528
Acne	0·961	0·643	0·534
Normal	0·507	0·453	0·522

TABLE II.—*Sebum Excretion (mg/10 cm²/3 h)*

	Active treatment Mean reduction	t	Probability
All	0·213	2·527	$P > 0{\cdot}05$
Acne	0·377	2·262	N.S.
Normal	0·085	1·580	N.S.
	Placebo treatment Mean reduction	**t**	**Probability**
All	0·185	2·041	N.S.
Acne	0·427	3·576	$P > 0{\cdot}01$
Normal	−0·030	0·336	N.S.

In conclusion, the evidence from this study is that topically applied 2% tetraynoic acid in ethanol + 20% of propylene glycol does not significantly reduce sebum excretion in acne patients. As this substance is effective orally, it is postulated that either the amount of tetraynoic acid absorbed by the skin is too small or that prolonged therapy is required before a significant result can be achieved.

Our thanks are due to Dr M. F. Corbett for the statistical guidance and to Dr H. Holgate of Roche Products Ltd. for kindly supplying the topical tetraynoic acid and placebo preparations.

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THE EFFECT OF TOPICAL TETRAYNOIC ACID ON THE SEBORRHOEA OF ACNE

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SUMMARY.—2 per cent tetraynoic acid with 20% propylene glycol in ethanol has no effect on the sebum excretion rate when applied topically in acne vulgaris. This contrasts with the slight but definite decrease in sebum excretion rate produced by 2% tetraynoic acid alone.

STRAUSS *et al.* (1967) showed that an inhibitor of cholesterol synthesis, eicosa 5–8–11–14–tetraynoic acid, would reduce the sebum excretion rate (SER) when given systemically to normal subjects and acne patients. Unfortunately, this drug is unsuitable for long-term administration and has been withdrawn from systemic use (Dr H. Holgate, Roche Products Ltd., personal communication) Since the drug appeared to be such a potent inhibitor of sebum production, we decided to try a topical preparation. With a 2% solution of tetraynoic acid applied topically in 7 acne patients for 8 weeks we noted a slight but significant decrease in sebum secretion (Burton and Shuster, 1970). Similar observations were made by Harroon *et al.* (1970).

In view of these encouraging results we have performed a larger study using the same preparation, 2% tetraynoic acid in ethanol, but with the addition of 20% propylene glycol, which is thought to increase the absorption of tetraynoic acid by the skin (Roche Products Ltd., unpublished data).

METHOD

Twenty volunteers, aged 16–28, all with acne, applied the solution twice daily to both sides of the forehead. Serial sebum excretion rates on each side of the forehead were determined by the method of Strauss and Pochi (1961), as modified by Cunliffe and Shuster (1969), on 3 occasions at weekly intervals before starting treatment and on 3 occasions at 2, 3 and 4 months respectively during treatment.

RESULTS

Nearly all the patients complained of a temporary mild erythema and " flaking " of the skin, and 1 patient discontinued treatment because of this. Another patient was lost to follow-up after the treatment was started. The results in the remaining 18 patients are shown in Table I. The mean sebum excretion rate during treatment, expressed as a percentage relative to the mean of the control values, was calculated for each patient, and the mean percentage was 98·7 ± SE 6%. The preparation was therefore without effect on the sebum excretion rate. In view of the well-known placebo effect in the treatment of acne vulgaris, no

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attempt was made to assess the clinical response objectively, but about half of the patients were pleased with the clinical results of treatment. There was no correlation between the patients' subjective assessments of their acne and the changes in sebum excretion rate during treatment.

TABLE I.—*Sebum Excretion Rates Before and During Treatment with 2% Tetraynoic Acid in Ethanol with 20% Propylene Glycol*

	Sebum excretion rates during control period ($\mu g/cm^{-2}/min$)			Mean of control values	Sebum excretion rates during treatment ($\mu g/cm^{-2}/min$)			Mean values during treatment
	1	2	3		2 months	3 months	4 months	
Mean	1·22	1·26	1·29	1·25	1·07	1·14	1·37	1·21
Standard error	0·12	0·14	0·13	0·13	0·10	0·16	0·23	0·13

The difference between the mean sebum excretion rates before and during treatment is not significant ($P > 0 \cdot 1$).

DISCUSSION

Two per cent tetraynoic acid in ethanol applied topically to the forehead skin causes a slight but significant decrease in the seborrhoea of acne (Burton and Shuster, 1970; Harroon *et al.*, 1970), but this effect is not seen when the preparation contains 20% propylene glycol. The reason for this is not clear, since it is believed that propylene glycol increases the absorption of tetraynoic acid by the skin (Holgate, personal communication, 1970).

In view of these findings and the powerful effect of tetraynoic acid in suppressing sebum production when given by mouth, further modification of the base seems desirable.

Thanks are due to Dr H. Holgate of Roche Products Ltd. for helpful advice and supplies of tetraynoic acid. Thanks are also due to Mrs D. Oxley for technical assistance, and to the Wellcome Trust and the Newcastle R.V.I. Research Committee for financial assistance.

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A STUDY OF THE EFFECT OF PREDNISONE AND AN ANTIHISTAMINE ON PATCH TEST REACTIONS

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SUMMARY.—A study was carried out to observe the effect of the administration of varying amounts of prednisone, as well as of the antihistamine Incidal, upon the patch test reaction in patients who had previously shown a positive or strongly positive reaction with different substances. A daily dose of 40 mg prednisone given to 12 patients was found to suppress the test reaction completely in 3 patients and to diminish it in 8; in 1 case there was no change. With a daily dose of 30 mg given to 18 patients, the reaction was suppressed in 4 and diminished in 6 patients; in 8 no difference could be detected. In the 16 patients who were given a daily dose of 20 mg prednisone, the test was completely suppressed in only 1, was diminished in 5 patients, and was unchanged in 10. In all of the 10 patients who were given the antihistamine Incidal (mebhydrolin napadisylate) no difference could be seen in the patch test reactions.

PATIENTS with a more or less acute allergic contact dermatitis are often treated with steroids or antihistamines, sometimes for quite long periods. Patch tests are often performed in order to discover the cause of the contact dermatitis, and the question then arises as to whether or not they should be done during the administration of such drugs, or postponed until treatment has been completed.

There are 2 possibilities. These drugs, steroids or antihistamines, may suppress the potentially positive patch test reaction, just as they suppress the patient's disease, so that the results of the patch test are not reliable. Or, it may be that the influence of the drug is not sufficiently strong to counteract the positive patch test reaction, despite its effect upon the disease itself, which commonly results from a contact with the allergen which is much less intense and prolonged than that provided in the patch test. If this is the case, the results obtained from the patch test may be considered valid.

A few studies have been carried out in an attempt to elucidate this problem, but all but 1 of them were made as long ago as 1951–52 when only cortisone and ACTH were investigated (Baldridge and Kligman, 1951; Nilzén, 1952; Sauer, 1951; Sulzberger *et al.*, 1952). Since then, only 1 study has been made of the effect of prednisone (O'Quinn and Isbell, 1969).

As it has been found that the effect obtained with prednisone varies significantly with the daily dose administered, we carried out a study in which 3 different daily doses were administered—20, 30 or 40 mg—and also included Incidal® (mebhydrolin napadisylate) as a representative of the antihistamines.

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METHODS AND MATERIALS

Patients were chosen for the study who were known to have had positive or strongly positive patch test reactions to various allergens, and who presented no contraindications to the administration of steroids. They were divided into 4 groups, with at least 10 patients in each group. In 3 groups an oral dose of prednisone (40, 30 or 20 mg) was administered daily, while those in the fourth group were given 200 mg of Incidal daily. Forty-eight h after treatment had been started, patch tests were performed with that substance to which each patient had formerly given a positive or strongly positive reaction. The tests were removed after 48 h; the results read after 48, 72 and 96 h, and compared with those previously obtained. The patients were given the drugs until the end of the study, i.e. the final reading of the results. Reactions were graded as follows: + = erythema; + + = erythema, oedema and papules; + + + = erythema, oedema, bullae, exudation.

RESULTS

The first group comprised 12 patients who were given 40 mg of prednisone daily. In 3 of them, patch tests which had been positive or strongly positive (++ or +++) became negative. In 8 patients a partial effect was noted: in 3 a +++ test became slightly positive (+); in 5 the reactions were diminished from +++ to ++ or from ++ to +. In 1 patient no difference could be noted between the 2 tests (Table I).

Table I.—*Patch Test Reactions*

No prednisone	40 mg prednisone daily	No. of patients
+++ or ++	—	3
+++	+	3
+++	++	3
++	+	2
+++	+++	1
		12

Table II.—*Patch Test Reactions*

No prednisone	30 mg prednisone daily	No. of patients
+++ or ++	—	2
+++	+	2
+++	++	2
++	+	2
+++	+++	7
		15

The second group comprised 15 patients who were given 30 mg of prednisone daily. Here there was a larger number of patients in which the prednisone was found to have had no effect upon the tests, with 7 of the 15 showing no change in reaction. In 2 patients the reaction, which had formerly been +++ or ++, became negative; in 6 it showed a diminution, more significant in 2, and only slight in 4 (Table II).

The third group comprised 16 patients who were given 20 mg of prednisone daily. In only 1 did the ++ test become negative, while there was no change at all in 10. In 5 there was a diminution of the reaction from +++ to ++ (Table III).

The fourth group comprised 10 patients who were given 200 mg Incidal daily. In 8 there was no change whatsoever, while 2 showed a very light difference, from +++ to ++ or from ++ to +.

As for the substances used in the patch tests, in the first group, of the 8 positive or strongly positive patch test reactions with potassium dichromate (0·5 %), 2 became negative. The third patch test reaction which became negative in this group was that with ointment of yellow precipitate of mercury 5 %. Of the 2 patch tests in the second group which became negative, 1 was with potassium dichromate and 1 with mercury. In the third group, only 1 patch test, with mercury, became negative; while, in the fourth group, none of the tests became negative.

TABLE III.—*Patch Test Reactions*

No prednisone	20 mg prednisone daily	No. of patients
++	–	1
+++	++	5
+++	+++	10
		16

DISCUSSION

Sauer (1951) found that a daily dose of 100 mg ACTH failed to affect a strongly positive patch test reaction to paraphenylenediamine or poison ivy. Similarly, the studies of Sulzberger *et al.* (1952) indicated that 150 mg of cortisone had no significant effect on strongly positive patch tests. Baldridge and Kligman (1951) found that even relatively large doses of cortisone failed to prevent the development of a delayed hypersensitivity to 2-4 dinitrochlorobenzene in guinea-pigs and this was confirmed by Nilzén (1952). Furthermore, the studies made by Baldridge and Kligman (1951) suggested that cortisone had no effect on the skin reactivity to test applications of 2-4 dinitrochlorobenzene after the animal had been sensitized, although Nilzén found that cortisone did influence the skin reactivity in these animals.

In 1969 O'Quinn and Isbell carried out a trial in which a dose of 40 mg prednisone was given to 20 patients 24 h before patch tests were performed with substances to which the patients had previously reacted strongly; in 6 patients the test became negative, while in the rest it remained positive.

The method used in our study differed from that of O'Quinn and Isbell as follows:

(1) The administration of prednisone was started at 48, and not 24, h before the patch tests were performed.

(2) Three different dosages of prednisone were administered to 3 groups (40, 30 and 20 mg) so as to compare the influence of the differing doses on the patch test reactions.

(3) The effect of the antihistamine Incidal was also studied in a separate group of patients.

The results obtained suggest that the smallest dose of prednisone given (20 mg) had almost no effect upon the reaction, while the higher doses of 30 mg and particularly 40 mg may cause a suppression or diminution of a previously positive or strongly positive test reaction, although this is not inevitably so, especially with the dose of 30 mg. Incidal was found to have essentially no effect on the patch test reactions.

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TREATMENT OF IDIOPATHIC HYPERHIDROSIS WITH IONTOPHORESIS OF TAP WATER AND POLDINE METHOSULPHATE

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SUMMARY.—Iontophoresis of tap water or of an anticholinergic compound (poldine methosulphate) was simple and useful in the treatment of idiopathic hyperhidrosis of the palms and soles. Axillary hyperhidrosis was not so easily controlled. Poldine was more effective than tap water; with increasing doses, systemic effects were produced.

Treatment was arranged easily in the Physical Medicine Department. It was suggested that, before resorting to surgery, iontophoresis is worth a trial in idiopathic hyperhidrosis.

TREATMENT of primary hyperhidrosis of the palms, soles or axillae is notoriously difficult. Failure of medical treatment is common, and there is reluctance to recommend surgery for a condition that is so frequently self-limiting. Surgery is, however, often very effective. Axillary hyperhidrosis may be well controlled by excision of sweat gland-bearing central axillary skin, although post-operative infection, scarring or hidradenitis may occur (Hurley and Shelley, 1967; Gillespie and Kane, 1970; Davis, 1971). Upper thoracic sympathetic ganglionectomy relieves hyperhidrosis of the hands, but may result in Horner's syndrome, pleural perforation, pleural effusion or phrenic nerve damage (Cloward, 1969; Greenhalgh *et al.*, 1971).

An alternative simple treatment of hyperhidrosis of the palms and soles was suggested by Levit (1968). This was iontophoresis of tap water. The attempts to introduce heavy metals into the body by iontophoresis (electrophoresis) were described in the eighteenth century (Burdick, 1966). The apparatus required for iontophoresis is a simple source of direct current of low voltage and low amperage. The skin resistance is lowest through the sweat ducts: hence the flow of current ions through the skin is greatest through the sweat ducts. A punctate skin pattern due to staining of sweat pores can be shown after iontophoresis of methylene blue (Papa, 1966).

The present study confirms the simplicity and usefulness of Levit's suggested treatment, and reports preliminary investigations on the iontophoresis of an anticholinergic compound, poldine methosulphate.

METHOD AND APPARATUS

The iontophoresis apparatus.—In the present study the apparatus used for iontophoresis was similar to that described by Levit (1968). Treatment was carried out in the Physical Medicine Department, or in the laboratory.

Electrodes.—Copper electrodes were used throughout (ideally platinum should be used instead of copper). An electrode, approximately the size of a hand or foot, was placed

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in the bottom of a shallow tray filled with tap water. The hand or foot to be treated was placed on the surface of the water so that the palms or soles were just submerged. Axillary electrodes (42 cm^2), shaped to fit the axilla, were well covered with gauze padding soaked in tap water.

Where only 1 side was treated an " indifferent " electrode, padded and soaked in tap water, was wrapped around the upper arm. If both sides were to be treated simultaneously, the circuit was completed by placing the second electrode either in the axilla or in tap water under the palm or sole.

Current.—Most patients could tolerate 15–20 mA for treatment of the hands or feet. For the axillae 10–15 mA were used (0·3 mA/cm^2 × area of axillary electrode). Painful sensation is produced at the anode by the liberation of acids (HCl when saline is used) and at the cathode by hydroxides (NaOH if saline is used).

Treatment was given for 15 min once or twice per week for 6 to 8 weeks.

TABLE I

	Years	
	Mean	Range
Age of onset	14	1–30
Age of attendance at outpatients	23	10–48
Duration of hyperhidrosis	7	0·5–20

TABLE II

Sites affected		Number of cases
	Feet	13
	Hands	12
	Axillae	10
	Trunk	2
Emotion stimulus	Feet	6
	Hands	9
	Axillae	6
	Trunk	2
Heat stimulus	Feet	5
	Hands	4
	Axillae	6
Exercise stimulus	Feet	3
	Hands	3
	Axillae	4

Anticholinergic compound.—The anticholinergic compound chosen for initial investigation was poldine methosulphate, as its possible side effects are well known. It has been used systemically since 1957 for treatment of peptic ulcers, and has been used topically to produce anhidrosis (Grice and Bettley, 1966).

For iontophoresis, poldine methosulphate, 0·05–0·075% in distilled water, was used at the anode.

The decrease in concentration following treatment was used as a measure of migration of poldine through skin. Poldine methosulphate was measured quantitatively by spectrophotometry (Langley *et al.*, 1963).

Method for measuring sweating.—Sweating was measured quantitatively by an electrical hygrometer (Bullard, 1962; Baker and Kligman, 1967). The presence of sweating was determined by starch-iodine (Kuno, 1956) by o-phthaldiadehyde (Juhlin and Shelley, 1967) and by measuring the galvanic skin resistance, using a sudorometer (d.c. resistance meter) similar to that described previously (Bettley and Grice, 1965).

Clinical features.—Twenty-two patients with idiopathic hyperhidrosis, 10 males and 12 females, were reviewed. The clinical features are shown in Table I, II. Three patients had a family history of idiopathic hyperhidrosis. The average age of onset was 13 years, and the mean age of attendance at outpatients was 23 years. The feet, hands and axillae were the sites most frequently involved (Table II). Emotional stimuli most commonly precipitated the attacks of hand sweating. As an example, a single case is reported in detail.

Case 1. A medical student aged 25 had severe idiopathic hyperhidrosis of the palms and soles since childhood. Sweating was not provoked by heat of exercise, but was much worse when he was studying or embarrassed. His father had similarly severe hyperhidrosis of the palms and soles, which started in childhood and cleared at the age of 20.

Conventional medical treatment improved 8 patients but had little effect on the remaining 13. Medical treatment included the usual oral anticholinergic compounds, sedatives, topical aluminium compounds, formaldehyde applications and topical poldine under polythene occulsion.

Thirteen patients were treated by iontophoresis. In order to have a quantitative comparison, 1 palm, sole or axilla was treated by one method and the opposite side was either left untreated or treated by another method. Tap water was used on the following sites: soles (9 cases), palms (6 cases) and axillae (4 cases). Poldine was used for soles (10 cases), palms (8 cases) and axillae (5 cases).

Where sweating was noticed to differ on the 2 sides, the more severely affected side was treated with the more effective method. A natural asymmetry of sweat delivery has been reported in 20% of people (Herrmann *et al.*, 1952).

RESULTS

Iontophoresis of either tap water or poldine methosuphate diminished sweating (Table III).

Tap Water

Tap water was less effective than poldine. Some patients with hyperhidrosis of the palms or soles were well controlled by tap water. One patient with extremely severe palmar hyperhidrosis was successfully treated by tap water. His treated hand remained dry, however, for only 2–3 weeks, and he needed further treatment at 2-monthly intervals. He was the medical student described previously.

Two children cleared completely for 2–3 months, although treatment had been given to only one side. Probably iontophoresis precipitated a natural remission. Relapses occurred in others 2–4 weeks after iontophoresis of tap water. Skin trauma resulting from iontophoresis was avoided if correct current dosage was employed, and any breaks in the skin surface were covered with grease before starting treatment.

Poldine

The hypohidrotic effect of poldine was more marked than that of tap water. The effect was very striking in 2 patients. They both failed to respond to tap water. Previous to treatment their hands constantly dripped sweat on the floor. After treatment their hands and feet were dry. One patient, 4 months later, had only minimal visible emotional sweating of the hands, feet and axillae. The other patient, however, relapsed after 3 weeks.

Two patients had a complete remission for 2–3 months after unilateral poldine had been given.

TABLE III.—*Effect of Iontophoresis on Sweating*

Site	Number of treatments	Sweat loss compared on 2 sides of the body (mg/cm^2/h)	
		Side treated with poldine	Untreated side
Palm	13	4·2	28·1
Palm	10	2·8	27·7
Palm	7	2·3	24·3
Palm	7	3·3	14·7
Palm	3	9·1	27·0
Palm	1	2·8	9·6
Foot	8	5·9	26·6
Axilla	4	1·5	5·6
Axilla	1	44·2	50·0
		Treated with poldine	Treated with tap water
Palm	3	1·1	6·6
Palm	1	3·6	6·0
Foot	2	2·1	4·2
Foot	1	2·4	4·8
		Treated with tap water	Untreated
Palm	17 (reading obtained 2 weeks after last treatment)	1·9	11·3
Palm	13 (reading obtained 4 weeks after last treatment)	7·8	11·2
Palm	11	2·6	8·8
Palm	8	3·1	5·8
Palm	8	5·0	5·6
Palm	5	7·7	16·8
Foot	6	13·5	15·8
Foot	5	2·9	4·6
Axilla	5	14·1	16·7
Axilla	3	16·0	24·2

TABLE IV.—*Decrease in Concentration of Poldine Methosulphate (Due to Migration Through Skin) Following Iontophoresis for* 15 *min*, 10–20 *mA*

Site of iontophoresis	Poldine methosulphate	
	Pre-treatment (mg/100 ml distilled water)	Skin absorption = post-treatment decrease in concentration (mg/100 ml)
Hand	75	4·2
Hand	75	4·1
Hand	75	2·7
Hand	75	8·8
Hand	75	1·7
Hand	75	1·7
Hand	28	3·4
Hand	13·3	2·7
Hand	96	2·7
Hand	10·6	1·8
	(mg/100 ml tap water)	
Hand	10·35	10·0

Poldine iontophoresis was most effective on the palms and least effective in the axillae. Four cases of hand and foot hyperhidrosis had remissions which lasted only 2–6 weeks. Three axillary hyperhidrotics were not adequately controlled.

Iontophoresis with poldine 50/100 ml in distilled water on 1 hyperhidrotic area per treatment (one palm, sole or axilla) caused no side effects and could be used with impunity. Larger doses or treatment of more than 1 area at a time was liable to cause side effects. The rate of disappearance of poldine resulting from iontophoresis was 1·7 to 18 mg/treatment/palm. The treatment dosages are shown in Table IV. To be effective the concentration of poldine had to be a great deal higher when tap water was used instead of distilled water. Concentration of up to 0·5% poldine could be tolerated with tap water, whereas side effects could be produced by $<0{\cdot}1\%$ solution in distilled water.

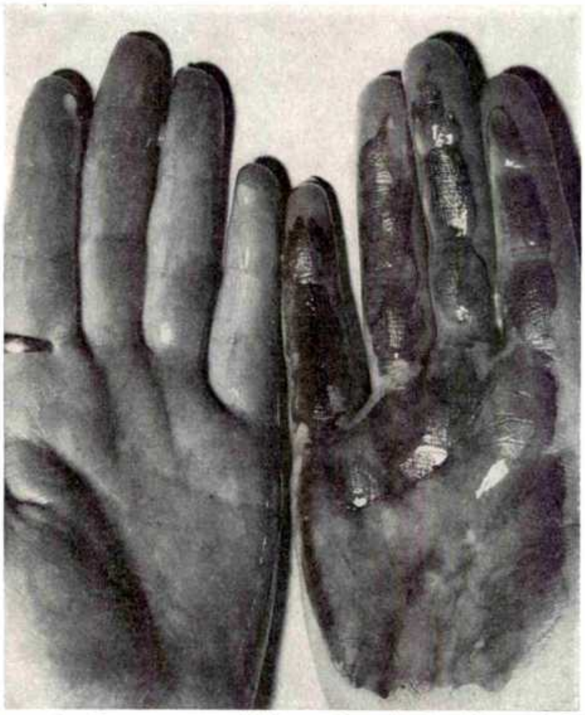

FIG. 1.—Three days after 7 treatments with iontophoresis of poldine to left hand; right hand untreated. Hyperhidrosis of right hand seen with starch-iodine; minimal sweating left hand.

The commonest side effect from percutaneous absorption of overdose of poldine was a dry mouth. Hypohidrosis of the untreated limb occurred on a few occasions, and mydriasis on 2 occasions. One patient complained of a gritty feeling of the eyes. The side effects occurred 15–30 min after treatment and lasted for 1–2 h.

CONCLUSION AND DISCUSSION

These preliminary findings confirm the usefulness of iontophoresis in the treatment of hyperhidrosis of the palms and soles. Most physical medicine departments have the apparatus and staff trained in its use.

Tap water produces no side effects and is easy to administer, provided the usual precautions are taken. Tap water may not be sufficiently effective, however, for treatment of hand and foot hyperhidrosis. Axillary sweating was never adequately controlled.

Poldine methosulphate was more effective, but was more difficult to administer. Hand and foot hyperhidrosis could always be controlled, but, as with tap water administration, the effect was often of short duration, and further courses of treatment were needed. In some cases long-lasting improvement (up to 3–4 months) were obtained; the treatment in these cases had possibly precipitated a natural remission.

Systemic side effects were produced by iontophoresis of poldine in high doses. When taken by mouth, there is a wide range of tolerance to poldine, side effects being produced by just under 10–100 mg (Lennard-Jones, 1961). The effect of topical poldine under polythene occlusion lasts for about 1 week (Grice and Bettley, 1966). Longer acting anticholinergics are being investigated, as are the effects of length of administration, current value and concentration of solution. Axillary hyperhidrosis was frequently not adequately controlled by poldine.

Solutions of poldine were not re-used after treatment, as the resulting concentration after treatment was not sufficiently predictable. Wahlberg (1970) showed that the maximum absorption of chromate (^{51}Cr) and sodium (^{22}Na) ions after iontophoresis was 43 and 8 times greater, respectively, than after local application. He used the rate of disappearance of the isotopes from the skin surface after iontophoresis to measure the rate of penetration of the ions through skin.

By measuring the uptake by the tissues of ^{32}P, O'Malley and Oester (1955) showed that increasing the concentration, current and duration of iontophoresis increases skin absorption. These findings were similar when using Ra^{131}I (Zankel, 1963) and ^{51}Cr (Wahlberg, 1970). ^{22}Na clearance, however, was little affected by duration or current. The effective concentration of poldine in distilled water was lower than that in tap water. This was due to the presence in the tap water solution of ions other than poldine methosulphate which would be attracted to the sweat ducts.

The cause of sweat inhibition following iontophoresis of tap water is said to be upper epidermal blockage of sweat glands; removal of the stratum corneum by stripping with Scotch tape relieves the obstruction. Larger current density affects deeper levels of the sweat apparatus (Gordon and Maibach, 1969.)

We thank Dr Pegum and Dr Almeyda for referring cases to us, the Physics Department and Miss Piercy for their help, the Physical Medicine Department for treating most of the patients, the Pharmacy and Elmer Perkins for measuring the poldine levels, the London Hospital and Schering Chemicals Ltd., Schering A.G. Berlin, for financial support, and Beecham Research Laboratories Ltd. for supplies of Nacton (poldine methosulphate).

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TREATMENT OF PSORIATIC NAIL DYSTROPHY

E. ABELL

SUMMARY.—A rapid, relatively painless method of treating psoriatic dystrophy of finger nails is described using a "Porton type" of needleless injector. Following treatment, 21 of 27 nails showed satisfactory return of normal appearance.

DESPITE the advances in treatment of skin disease in recent years, no reliable and reproducible methods have been devised to treat nail dystrophies, except those caused by fungal invasion. The suppressive action of the halogenated corticosteroids on cutaneous psoriasis is well established despite their many shortcomings. However, no satisfactory means of delivering these drugs to the nail matrix has yet been found other than by the systemic route.

In 1962, Gerstein demonstrated that triamcinolone acetonide suspension injected into the nail matrix area of psoriatic nails produced a useful improvement in 4 of 7 cases treated. Using a conventional syringe and needle technique, the procedure required local digital "ring" block anaesthesia.

Needleless jet injectors (Porton Gun) have been used in dermatology by Moynahan and Bowyer (1965) and Verbov and Abell (1970), and possess the considerable advantage of giving rapid and relatively painless intralesional therapy. This instrument has been used to treat psoriatic nail disorders in which matrix involvement is considered to be responsible.

MATERIALS AND METHOD

Seven patients, 6 showing cutaneous psoriasis in addition to the nail dystrophy, were treated by deep intradermal injections of the matrix area of affected nails, through the posterior nail fold by means of a needleless injector. The clinical details, treatment and results are summarized in Table I.

Sterile triamcinolone acetonide solution diluted to a strength of 5 mg/ml was used, and the instrument was sterilized using 0·5% chlorhexidine in 70% industrial spirit, as described by Verbov and Abell (1970).

Treatment consisted of injections on 3 occasions at weekly intervals, 1 to 10 (average 5) injections of 0·1 ml being given to each of 27 fingers. Follow up varied from 3 to 10 months, average 6·7 months. A total of 27 nails was treated.

RESULTS

In 5 of the 7 cases complete return of normal nail resulted. A single nail of 3 treated (Case 6) showed only marginal improvement, and all nails failed to show improvement in Case 6.

Abnormal nails left untreated in Cases 1, 2, 3 (treated in two series) showed no sign of spontaneous improvement during the follow-up period. In Cases 5

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TABLE I

Case no.	Sex	Age	Clinical state and duration		Clinical involvement of nails	No. of nails treated	Maximum dose/nail	Result
1	M	35	Nails only	4 months	Uniform pitting all nails	3	4 mg	All treated nails complete clearance, but relapsed after 4 months. No change in untreated nails
2	F	44	Flexural psoriasis Nails	26 years 2 years	Severe pitting and/or onycholysis of 8 nails	4	4 mg	Pitting cleared only in treated nails without change in onycholysis. Pitting relapsed in 1 at 10 months
3	M	52	Generalized psoriasis Nails	30 years 1 year	Irregular pitting and gross onycholysis all nails, left hand (right arm amputated)	3	9 mg	Clearing of pitting in all and onycholysis in 2. Marginal onycholysis in third remained.
						2	10 mg	Complete clearance of pitting and onycholysis.
4	F	69	Scalp Nails	25 years 3 months	Severe onycholysis left 3; gross destruction left 4; elevated onycholysis left 5	3	7·5 mg	Complete clearance left 3, 4 Slight improvement left 5
5	F	44	Generalized psoriasis Nails	30 years 6 years	Uniform pitting, 3 nails, 1 with onycholysis	3	4·8 mg	Pitting and onycholysis cleared in 3 treated nails. Pitting relapsed in 1 at 5 months, and involved 3 others during follow-up
6	M	42	Generalized psoriasis Nails	2 years 2 years	Gross pitting with destruction and onycholysis, 8 nails	4	7 mg	No change, treated or untreated nails
7	M	62	Generalized psoriasis Distal arthritis Nails	20 years 9 months	Gross destructive changes, 4 nails; a single nail with marginal onycholysis	5	10·6 mg	Reformation of the 4 grossly affected nails. Onycholytic nail deteriorated and 2 others became similarly involved. Symptomatic improvement of arthritic pain in treated fingers

and 7, untreated normal nails became involved, those of Case 5 being subsequently successfully treated together with a nail showing relapse of pitting after 5 months. Altogether, relapse occurred in 5 nails after an average duration of 5·4 months (range 4–10 months).

The return of normal nail was seen in all successful cases within 8 weeks of onset of therapy. Matrix nail dystrophy from mild pitting to gross nail disorganization is followed by reformation of normal-appearing nails. Fig. 1 shows that onycholysis may also clear; in Case 4 this was still absent 6 months later (Fig. 2).

No serious complications of the procedure were seen; the injections, completed with slight discomfort within 1 min, produce a white swelling of the posterior nail fold, which subsides during the next hour. Minor haemorrhage occurred on occasions, but was easily controlled by local pressure. The fingers may remain slightly tender for 24 h, but no patients experienced difficulty in pursuing their normal activities at work. Penetration of injections at this site may be incomplete; the dosage given in Table I represents the maximum available. Sepsis was not seen; atrophy of the nail fold was either slight or absent, and when seen, did not persist beyond 3 months.

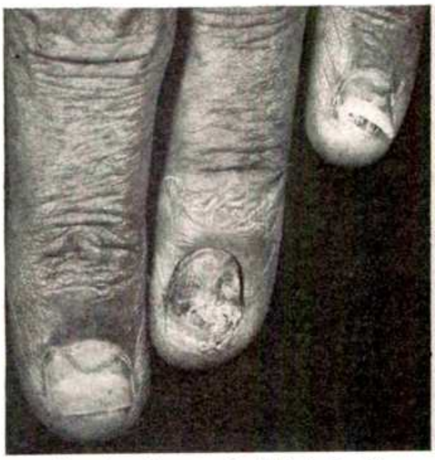

FIG. 1.—Case 4—Left 3, 4, 5 nails before treatment.

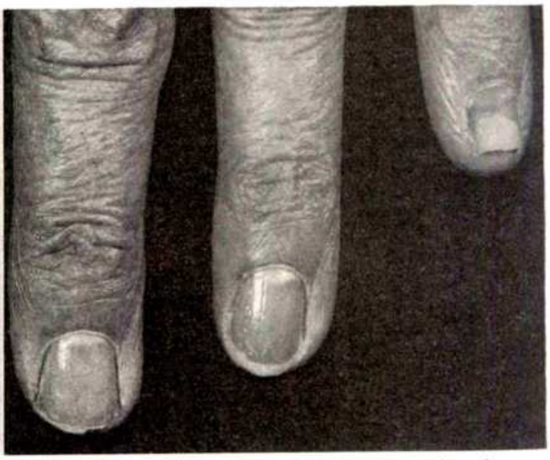

FIG. 2.—Case 4—Left 3, 4, 5 nails 6 months after treatment.

DISCUSSION

Despite occasional improvement after use of halogenated topical corticosteroids under occlusion or superficial x-ray therapy, and the tendency for spontaneous resolution, the treatment of nail psoriasis remains unsatisfactory (Samman, 1965, 1966). Gerstein's technique suffers greatly from the limitations imposed upon it by the necessity for local anaesthesia. In dealing with psoriasis in any site, relapse must be anticipated, and it is therefore important that treatment should be quick, effective and easily repeatable. The needleless injector appears to fulfill these criteria.

The effective duration of treatment does not suggest that systemic effects are likely to arise, even with repeated injections.

Matrix abnormalities of the nail might be expected to improve, but onycholysis, considered to be due to nail bed involvement, might also be affected. Improvement, when seen, was gradual and appeared to improve with the advancing new nail, which suggests that the quality and/or rate of matrix function might also play a part in this condition.

I am grateful to Dr Peter Borrie and Dr D. D. Munro for permission to treat their patients; also to Miss J. Bailey for supply of sterile fluids and to Mr Tredinnick and Miss Dorrington of the Department of Medical Illustration for the photography.

The injector is available from Schuco International Ltd.

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PENETRATION AND DOSE OF INJECTIONS WITH THE PORTON JET INJECTOR

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SUMMARY.—Factors concerned in the performance of the Porton jet injector were investigated. Changes of settings produced wide variation in the percentage of the ejected dose that actually reached its target, as well as in the depth of injection. It is concluded that, while this instrument is useful for rapid work, it leaves a great deal to be desired in reproducibility and accuracy of the delivered dose. These findings have a bearing on the use of the Porton jet injector for multiple skin injections and in procedures such as mass immunization.

The Porton jet injector is an instrument designed to inject between 0·1 and 1·3 ml of a solution either intradermally or intramuscularly. The depth of penetration is varied by changing the diameter of the nozzle through which the jet emerges, or by altering the distance between the nozzle and the skin surface. For this purpose 3 spacers and 2 nozzles of 0·006 in (150 μm) and 0·008 in (200 μm) diameter are provided.

Needleless injectors are useful when large numbers of injections, each of similar size, are to be administered to the same patient (Moynahan and Bowyer, 1965) and have been widely employed in mass immunization programmes. The method is quick, causes less discomfort than a conventional needle and is claimed to deliver a reproducible dose.

Information concerning the depth of penetration and amounts which are actually deposited are not available. White (1969), Moynahan and Bowyer (1965) and Figge and Barnett (1948) showed x-ray pictures of radio-opaque material in human limbs, but depth of penetration is difficult to assess with this technique.

Having used the instrument for clinical purposes, particularly to inject small amounts of steroids intradermally, we noticed an obvious variation in the dose delivered to adjacent sites. The present study was therefore undertaken to measure the depth of penetration and the volume of material injected. The number of ejections required to remove a solution already in the apparatus, and to replace it by a second, was also investigated.

MATERIALS AND METHODS

Abdominal skin obtained at post-mortem was used throughout the experimental work, except where otherwise stated.

The variation in amount of solution delivered was determined by ejecting distilled water into weighed tubes and reweighing. The weights were measured to 0·1 mg.

The depth of penetration was studied by injecting 0·1% toluidine blue in water. The centre of the stained region was divided into 2, and the extent of penetration was

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measured using a dissection microscope and an eyepiece graticule where the depth was less than 1 cm; a rule with 1 mm divisions was used when the penetration exceeded this. The size of the stained areas was studied at intervals after the injection and no evidence of diffusion was found. The stain was apparently firmly bound to those tissues with which it came into immediate contact.

The volume injected into the skin was determined radioisotopically. ^{131}I was added to 0·1% toluidine blue and injected into the skin. Any solution remaining on the surface was wiped off; the stained areas were dissected out and the radio-activity measured by a gamma spectrometer.

The rate of dilution was measured by placing a dye solution in the injector and diluting this with distilled water in the manner recommended by the manufacturers. The optical density of the solutions was read and the concentration determined from a previously-made calibration curve.

RESULTS

The variation in the amounts delivered is shown in Table I. Each determination is the mean of 10 readings.

TABLE I.—*Range of Volume Delivered by Injector at Certain Settings*

Injector set to deliver (ml)	Nozzle	Volume delivered (ml)	Coefficient of variation (%)	Difference between volume delivered and volume set to be delivered (ml)
0·05	Small	0·068	2·1	0·018
0·05	Large	0·070	1·1	0·020
0·10	Small	0·120	1·1	0·020
0·20	Small	0·221	1·1	0·021
0·20	Large	0·219	0·8	0·019
0·30	Small	0·321	0·8	0·021

Fig. 1 shows the influence of the size of nozzle and the distance of the nozzle from the skin surface on the depth of penetration when the injector is set to deliver 0·1 ml. Fig. 2 shows the penetration of 0·1 ml of dye into the skin, using the intermediate spacer. The picture shows the comparative penetration using the large and small nozzles. When the injector is set to deliver 0·05 ml, using the large nozzle, penetration frequently does not occur when using the long spacer. Injections from the large nozzle delivering 0·2 ml in contact with the skin surface may penetrate the full thickness of the abdominal wall, except when there is a well-developed layer of fat. With larger volumes the depth of penetration is in excess of 2·5 cm.

The penetration of the radio-active dye solution showed that the variation in the volume of the solution entering the skin was less than 12% when volumes greater than 0·1 ml were delivered, using the large nozzle. About 50% of the dose entered the skin when 0·1 ml was ejected from the large nozzle. Only 30% of the liquid penetrated when the machine was set at 0·05 ml, and the most consistent results were obtained using the short spacer.

The results of tests with the small nozzle showed that the dose penetrating is much more variable. The variation obtained when the machine was set to deliver 0·05 ml was between 0 and 25% of the volume ejected. When set to deliver 0·1 ml, the short spacer gave the least variation when assessed visually. The radio-active results were obtained only from this setting and showed that about

25% penetrated. The variation was between 12% and 35% of the volume ejected.

The washing of dye from the machine showed that the concentration decreased by 43% at each firing. Dye could be introduced into the reservoir on firing, as was evident by its presence.

DISCUSSION

The variation in the volume of solution ejected shows that the Porton jet injector behaves very well as a means of delivering small volumes of similar size. On the other hand, this jet injector is less satisfactory in its actual ability to deliver accurate volumes into the skin.

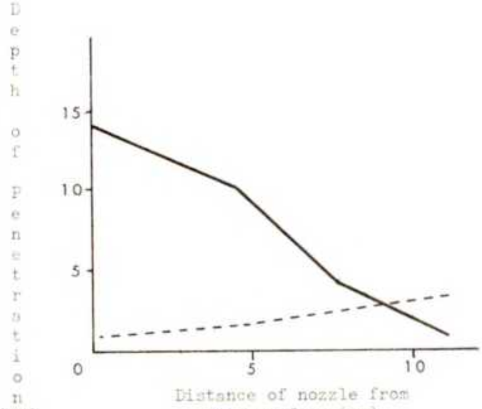

FIG. 1.—Variation in depth of penetration with changes in distance between nozzle and skin surface.

—— 0·008 in diameter nozzle
– – – 0·006 in diameter nozzle

These studies show that the depth of penetration varies with the distance of the nozzle from the skin surface. In Fig. 1 the variation in the depth of penetration is consistent for each of the nozzles. The different behaviour of each of the

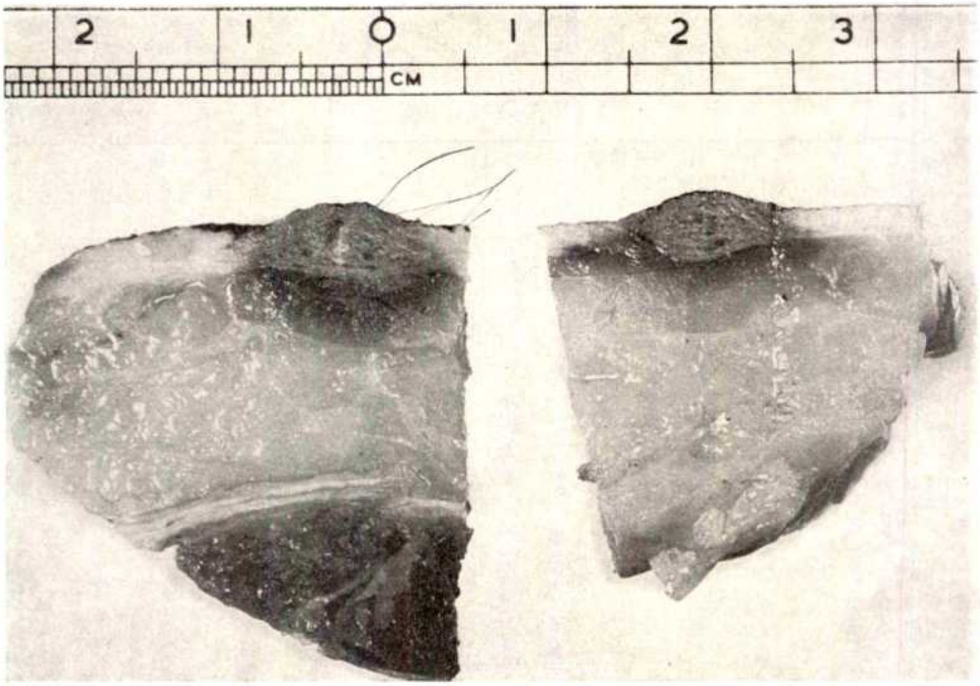

FIG. 2.—Penetration of dye in abdominal skin, using intermediate spacer. Injector was set to deliver 0·1 ml using 0·008 in nozzle for skin sample on left, and 0·006 in nozzle for sample on right of picture.

nozzles is unexpected and suggests that more work should be done on the effect of the size of the nozzle and the manner in which it affects penetration.

It is also evident from this work that the volume of solution entering the skin is less than the volume the machine is set to deliver. This point has been ignored in a number of reports where it was assumed that the total volume delivered is completely deposited in the skin. It has also been assumed that the volume setting is accurate, whereas we have been informed by the makers that the zero setting on the machine is frequently inaccurate and may be out by as much as 0·05 ml. Both these factors should be taken into account, and if it is necessary to know the total volume injected, more conventional procedures should be used.

It must also be noted that, even if a table of values relating dose to setting were available with the Porton injector, this would be of use only as a guide. The variation in volume entering the skin, particularly when the volume is small, would not enable one to use this apparatus to administer accurately known amounts.

A further variable arises from differences in behaviour of the injector at different sites, since the degree of penetration is undoubtedly affected by the mechanical properties of the skin and the underlying tissues. This was evident from studies made on the skin of the thigh and scalp as well as our studies on abdominal skin.

The depth of penetration of the injections using abdominal skin led us to consider whether the injector might cause damage to the periosteum when used on the scalp to treat alopecia areata. Use of the injector on the scalp of cadavers has shown that volumes of up to 0·2 ml, using the 0·008 in aperture in contact with the skin, do not penetrate sufficiently deeply at this site to cause such damage.

For the dermatologist, depth of penetration of the substance injected is of great importance. Using the nozzle with the smaller aperture, which is essentially designed for injection into the dermis, the injected material was in fact found at the dermal level. However, depth of injection using the nozzle of greater diameter varies in various sites of the body, and it is probably wise to avoid using this attachment for injections of regions with underlying structures which could be damaged in this way.

Our studies also showed that the concentration of a solution remaining in the instrument is reduced to only less than 1% after 10 ml have passed through it. The makers recommend that 3 volumes of 1·3 ml are sufficient to charge the machine and displace solutions already present.

Having stressed the inaccuracies of the injector it should be pointed out that in most clinical uses of the injector the dose administered need not be quite exact.

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Comment

Br. J. Derm. (1972) **86**, 87.

ANTIMETABOLITES AND PSORIASIS

All effective antimetabolites share two features—they inhibit DNA synthesis, and they exhibit systemic toxicity. Those tissues with the highest mitotic index are the most vulnerable to the adverse effects as they are at the same time most sensitive to the benefits. In psoriasis there is considerable hyperplasia of the epidermis, although it is still not clear whether this is of dermal or of epidermal origin (Christophers and Braun-Falco, 1970). These abnormalities are also found in clinically uninvolved skin (Born and Kalkoff, 1969), where in addition to increased DNA synthesis and thymidine incorporation, enhanced activity of the pentose-monophosphate shunt has been found (Gordon and Johnson, 1967). Abnormalities in protein metabolism have also been observed in psoriatics (Zaruba *et al.*, 1969), and immunoelectrophoresis of aqueous extracts of epidermis from such patients has suggested the presence of an unusual globulin (Sellei *et al.*, 1970). The enhanced transit time through the epidermis may upset the incorporation of histidine into precursors of keratohyalin, since this appears to be genetically programmed on a fourteen-day cycle (Voorhees *et al.*, 1968). Abnormalities in purine and pyrimidine metabolism have been observed in patients with psoriasis, but not with great consistency (de Bersaques, 1967), although the normal salvage mechanism for pyridine bases may be impaired (Baden, 1967). Studies in rats, mice, and rabbits suggest that there is a correlation between the growth rate of a tissue and the efficiency of a *de novo* pathway of synthesis of pyrimidine from orotic acid (Sköld, 1960). These diverse data emphasize the gaps in our knowledge of the nature of psoriasis, and the differences—if any—between this disease and normal regeneration on the one hand, and frank neoplasia on the other.

The treatments of psoriasis reflect our uncertain grasp on the fundamentals of the pathology. Arsenic had the advantages of remarkable efficacy and the ease of oral administration, but the long term risks of cutaneous and systemic cancers are now well known. The element interferes with protein and nucleic acid synthesis, amongst other effects, and its benefit may result solely from its action as an antimetabolite. Perhaps there is still a limited place for arsenic in the treatment of elderly patients, as the latent period before the appearance of neoplasms averages 20 years (Neubauer, 1947), although it can be as short as 13 years (Sommers and McManus, 1953). Similarly, the action of mercury and ammonia ointment may be due to the poisonous effect of the metal selecting the psoriatic cell population in preference to the normal cells. The undoubted beneficial effect of dithranol and coal tar is partially due to the inhibition of mitosis, although additional actions are likely (Fry and McMinn, 1968). This work has been confirmed and extended, and both glucocorticosteroids and occlusion alone will also depress the mitotic index of psoriatic lesions (Baxter and Stoughton, 1970). The antimitotic activity of corticosteroids seems to be related to their therapeutic efficiency both by the oral and topical routes (Fisher and Maibach, 1971). The more powerful anti-inflammatory glucocorticoids also suppress amino acid incorporation and glycogen synthesis in rat liver and muscle (Peters *et al.*, 1970). Ultra-violet light is also a well tried modality in the management of psoriasis, and this produces depression of DNA synthesis, and immediate damage to the DNA present in the skin at the time of exposure (Tan and Stoughton, 1969). A stimulation of DNA synthesis by u.v. light has been demonstrated in hairless mouse epidermis, and this may be a repair mechanism (Epstein *et al.*, 1968) in response to the injury produced.

It is quite clear therefore that powerful poisons have been used for centuries in the treatment of psoriasis, and this generation is not new in this respect. Perhaps some of the outcry against Methotrexate would be abated by a better understanding of the toxic achievements of the past. The value of Methotrexate in psoriasis is now well established, and so is the risk (Baker and Dahl, 1969). Liver disease is the major problem with

Methotrexate, even in the absence of ethanol consumption (Dubin and Harrell, 1970), although the background is not fully understood. The possible occurrence of a liver abnormality in psoriatics as part of the disease process itself has not yet been evaluated. As with psoriatic arthropathy, there may be a sub-group of patients at extra risk because of the systemic spread of their disease. Topical use of Methotrexate was advocated (Fry and McMinn, 1967), and then found wanting (Comaish and Juhlin, 1969), so this method of reducing toxicity cannot be used generally. It is quite possible that metabolic alteration of Methotrexate is necessary to obtain maximal effect upon psoriasis, and derivatives have been identified in urine, although they have not been characterized (Henderson *et al.*, 1965). Impurities and other variants between batches may also have clinical significance (Stanley *et al.*, 1968). For some time it was thought that the action of Methotrexate was via the competitive inhibition of dihydrofolate reductase alone. An inhibitory effect upon thymidylate synthetase has also been reported, but at concentrations of the drug which are probably rarely if ever obtained in the treatment of psoriasis (Borsa and Whitmore, 1969). Additional sites of action include competition for the folic acid cell entry mechanism (Johns *et al.*, 1963), and inhibition of the conversion of exogenous folic acid to compounds with citrovorum factor-like activity (Condit, 1961). It has been shown recently that tissues contain proteins with affinity for Methotrexate, which are not enzymic in nature, and which are of higher molecular weight than dihydrofolate reductase (Newbold and Harding, 1971; Kaufman and Pierce, 1971), so the old simplistic viewpoint is no longer tenable. Of interest in the evaluation of these other binding proteins is the report that the liver enzyme can recover from inhibition by Methotrexate by separation from the drug (Stanley *et al.*, 1969) The absorbed Methotrexate is then free to damage the liver parenchyma, or any other tissue with binding proteins. Perhaps these compounds explain the long persistence of the drug, as well as the pattern of tissue toxicity. Other harmful effects of Methotrexate include mouth ulcers, marrow depression, damage to the intestinal mucosa (Trier, 1962), the precipitation of gout (Martin *et al.*, 1967), a characteristic pulmonary syndrome (Clarysse *et al.*, 1969), and reactivation of acute inflammation in a manner resembling photosensitivity (Möller, 1969). Efforts to alter the total dosage of Methotrexate by more rational regimes are a step in the right direction (Nyfors and Brodthagen, 1970; Halprin *et al.*, 1971), but it is apparent that a better treatment is needed.

Hydroxyurea has now been in use for over 5 years, and there is no doubt that it can be effective (Leavell and Yarbro, 1970). It has a wide margin of safety and it is considerably less toxic than Methotrexate. Unfortunately it is less effective in clearing the severe and chronic cases for whom an antimetabolite would be considered, so it must be concluded with regret that only a small fraction of the psoriatic population is likely to benefit from hydroxyurea. A few reports have appeared of excellent results with the xanthine oxidase inhibitor allopurinol, which is of interest in view of the high uric acid levels found in many patients (Bradford and Hrehorovitch, 1969). Toxicity is minimal, and the drug may be stopped without severe rebound (Viglioglia *et al.*, 1970). Other antimetabolites, which have been used sporadically without full trial or comparison, include triacetyl azauridine (Calabresi and Turner, 1966), azaribine (Vogler and Olansky, 1970), and busulfan (Möller and Waldenström, 1970). One feature of all these reports is the claim that the compound in question is less toxic than Methotrexate. The topical application of 5-fluorouracil under occlusion produces erosion and subsequent clearing of psoriatic plaques (Nurse, 1963), without the Koebner response that might be expected in view of the injury produced. Maibach (1971) has used both 1% and 5% 5-fluorouracil in propylene glycol, and is impressed by the results despite the pain and the initial ulceration. The nitrogen mustard Mechlorethamine has also proved successful as a topical application, despite some risk of allergic contact dermatitis (Mandy *et al.*, 1971).

To conclude, the future of therapy in psoriasis looks more promising today than it has ever done before. And this is without any major breakthrough in our basic understanding of the background of the disease. We can be certain that antimetabolites will continue to be important in the future, but topical application will probably be refined to reduce mess, and systemic administration may not be feasible because of the risks of toxicity. It is likely that future agents will be more selective, and their specificity will be based upon a knowledge of the biochemical lesions in psoriasis. Methotrexate seems bound to be superceded, despite its interest and efficacy, and the same fate probably awaits the corticosteroids—for this entity at least.

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Case Reports

Br. J. Derm. (1972) **86**, 91.

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ACROKERATOSIS VERRUCIFORMIS OF HOPF WITH STEATOCYSTOMA MULTIPLEX AND HYPERTROPHIC LICHEN PLANUS

JULIAN VERBOV

SUMMARY.—The case is reported of a woman aged 50 with acrokeratosis verruciformis of Hopf. The patient's father and a sister were said to have similar lesions. The patient also had steatocystoma multiplex in the presternal region and hypertrophic lichen planus over the elbows.

ACROKERATOSIS verruciformis of Hopf (a.v. of H.) is a genetically-determined skin condition characterized by indolent papules resembling plane warts on the

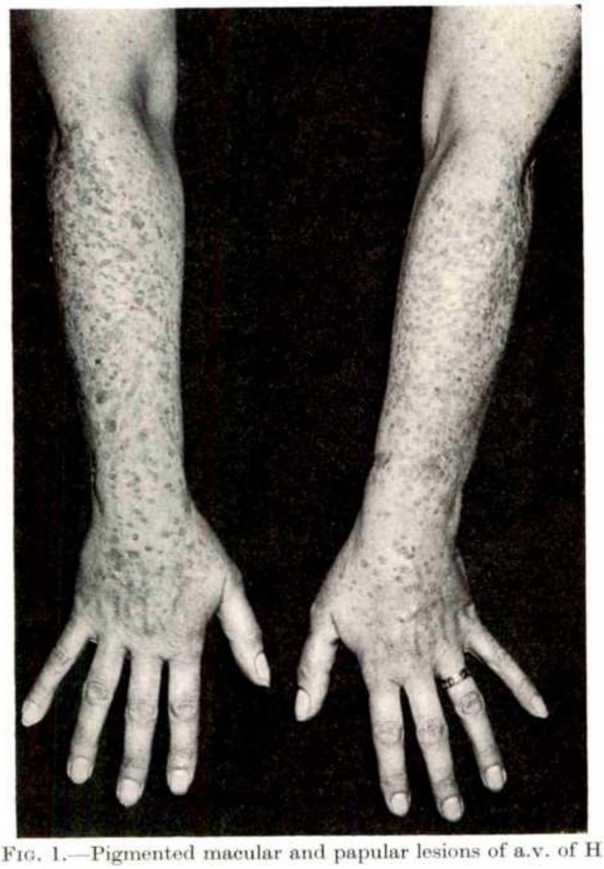

FIG. 1.—Pigmented macular and papular lesions of a.v. of H.

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dorsa of the hands and feet and often over palms, soles, knees, forearms and elsewhere. The nails may be thickened and white (Rook *et al.*, 1968). It usually shows an autosomal dominant form of inheritance (Niedelman and McKusick, 1962).

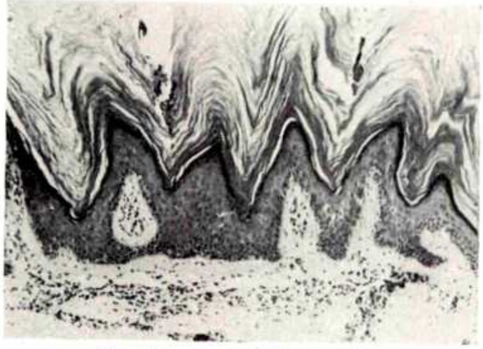

FIG. 2.—A.v. of H. (×90.)

CASE REPORT

Mrs A. C., housewife, aged 50. Since childhood she has had raised brown lesions over the backs of the hands and on the shins, with slow spread over the limbs. She continues to develop fresh lesions, and older lesions are becoming more prominent. She has also noticed elevated, purplish lesions over the elbows since 1963, and smooth, flesh-coloured papules over the midline of the chest have been present for about 20 years.

Family history.—She says that her father had similar pigmented lesions over the arms, and 1 of her 4 sisters, aged 46, has recently developed similar pigmented lesions.

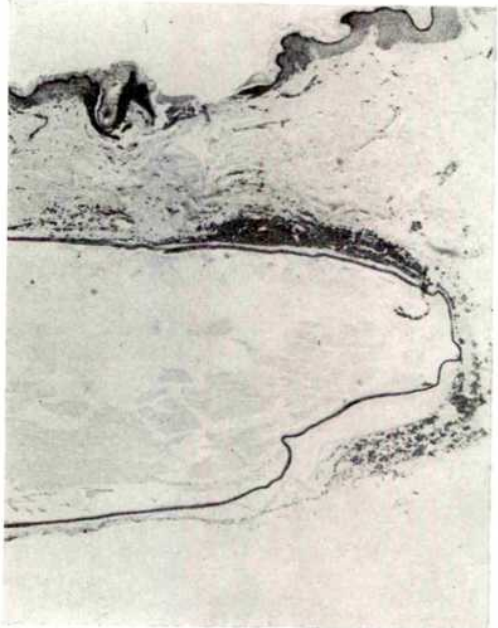

FIG. 3.—Small cystic chest lesion—histology of steatocystoma multiplex (Dr K. V. Sanderson). (×50.)

Clinical examination.—Discrete pigmented macules are present over the extensor aspect of the arms and over the lower legs; some elevated lesions look like seborrhoeic keratoses (Fig. 1). The trunk is almost free of lesions, but there are a few typical seborrhoeic keratoses. A few flat papules are present over the left palm, and the soles are hyperkeratotic. There are nodular hyperkeratotic lesions of lichen planus over the elbows, more marked on the right side. There is a group of small, discrete, smooth, cystic lesions over the presternal region.

Investigations.—Hb, WCC, ESR, x-ray of chest, barium swallow and meal—all normal.

Histology.—1. *Flat papule from dorsum of hand (present since childhood) and a raised papule from upper arm (of recent origin).* Both sections show considerable hyperkeratosis, a thickened granular layer and acanthosis. The epidermis shows circumscribed elevations, and the features fit a diagnosis of a.v. of H. (Fig. 2). There is no vacuolation in epidermal cells and no parakeratosis.

2. *Elbow papule.*—Shows lichen planus.

3. *Presternal lesion.*—Histology shows a cyst in the mid-dermis, with a sebaceous gland lobule forming part of its wall (Fig. 3).

DISCUSSION

Niordson and Sylvest (1965) described a patient with Darier's disease, Hailey-Hailey syndrome and a.v. of H. They considered it probable that all 3 disorders were variants of a single disease. However, regarding Darier's disease and Hailey-Hailey, genetic studies (Svendsen and Albrectsen, 1959; Raaschou-Nielsen and Reymann, 1959) suggest that they occur independently in affected families and are not known to be genetically linked.

Others have also considered that a.v. of H. and Darier's disease may not be separate entities. Thus, Herndon and Wilson (1966) considered that they are determined by a single dominant gene with variable expressivity, and Krause and Ehlers (1969) felt that it was no longer justifiable to separate the 2 disorders. Herndon and Wilson (1966) described a family in which 7 members had a.v. of H. and another 1, possibly 2, had Darier's disease. Niordson and Sylvest (1965) also mentioned a.v. of H. occurring in relatives of patients with Darier's disease; Waisman (1960) and Penrod *et al.* (1960) described a.v. of H. occurring in patients with Darier's disease.

Darier's disease and a.v. of H. are probably not genetically distinct. It may be that a.v. of H is an abortive form of Darier's disease, as Waisman (1960) suggested, in which only non-dyskeratotic lesions have evolved. This being so, such hyperkeratotic lesions may be seen in typical Darier's disease, or may occur alone as in the patient described in this paper.

It is interesting but coincidental that this patient should have chronic hypertrophic lichen planus affecting the elbows. The presence also of steatocystoma multiplex, an uncommon condition, is of greater interest, as this condition is inherited as an autosomal dominant character in many cases.

I should like to thank Dr P. F. Borrie for allowing me to publish this case, which was presented at the Section of Dermatology, Royal Society of Medicine, April 16th, 1970.

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ECCRINE SWEAT GLAND CARCINOMA WITH METASTASES

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SUMMARY.—A case of metastasizing eccrine sweat gland carcinoma is presented. It developed on the thigh, which is a highly unusual site. The course was consistent with the recognized poor prognosis.

SWEAT gland carcinoma is rare, with a high incidence of metastatic spread; Of 39 cases reviewed in 1967 (Miller, 1967), 28 eventually had metastases. It is unusual for this tumour to appear on the lower limbs. The patient described below had sweat gland carcinoma on the left thigh, with regional metastases 15 months later. She died of mediastinal and lung metastases 3 years and 4 months after the onset of the primary lesion.

CASE REPORT

A woman aged 59 was first seen in January 1966, with a bluish nodule on the lower part of the anterior aspect of the left thigh, which had been present for 2 months. It was 2 cm in diameter, with a smooth surface, and tender (Fig. 1). The inguinal nodes were not palpable. Histological examination showed a nonencapsulated, peripherally infiltrating, anaplastic, malignant, epithelial tumour, with fairly frequent mitoses. At the edge of, and caught up in the tumour, were normal eccrine sweat glandular acini showing apparent transition to tumour cells, some of which contained P.A.S.-positive and diastase-sensitive globules. The lesion was thought to be a malignant eccrine sweat gland tumour (Fig. 2, 3). Local excision was widely complete.

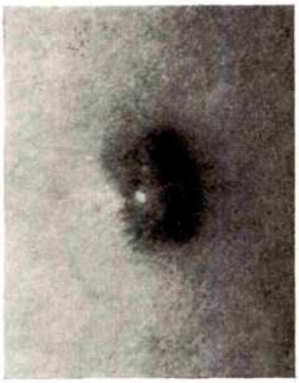

FIG. 1.—Nodule on lower part of left thigh.

In May, 1967, at follow-up, she was found to have enlarged inguinal nodes. A block dissection was carried out, and the histological changes in the nodes were identical to

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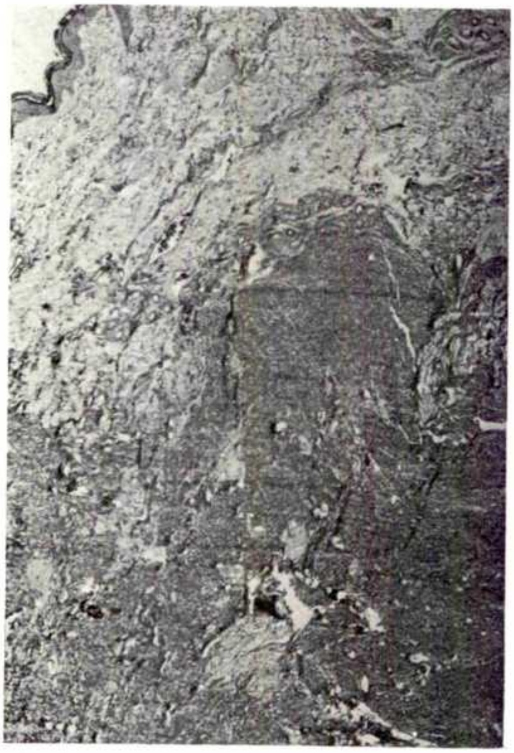

FIG. 2.—L.P. Poorly encapsulated tumour in the deep dermis and subcutaneous tissue with foci of recognizable sweat glands. (H. and E. ×140.)

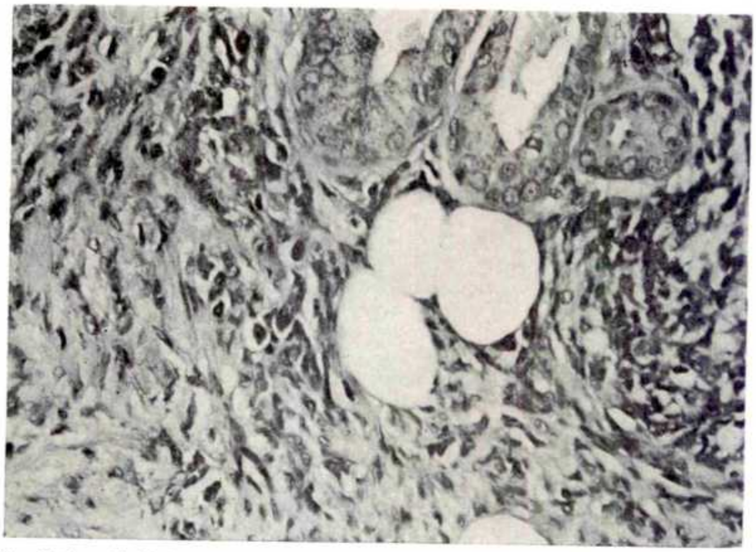

FIG. 3.—M.P. Field from centre of lesion showing acinar structures amidst anaplastic tumour cells. (H. and E. ×350.)

those of the primary lesion. When reviewed in March, 1969, she had dyspnoea on exertion and swelling of the face and neck, for 2 weeks. The neck veins were distended and the veins on the upper chest and back prominent. X-ray of the chest showed a round mass projecting from the right side of the mediastinum, with a smaller opacity lateral to it at the level of the aortic arch. Tomography confirmed the presence of enlarged mediastinal nodes compressing the trachea. A single dose of deep x-ray therapy aggravated her dyspnoea. She deteriorated rapidly and, soon after commencing therapy with cyclophosphamide, she died in April, 1969.

At post mortem there was gross enlargement of the tracheo-bronchial group of nodes, with invasion by a fleshy haemorrhagic tumour, ulcerating into the bronchial mucosa. There were also 2 well-defined tumour nodules 4 cm in diameter in the parenchyma of the upper lobe of the right lung. The superior vena cava was occluded by a thrombus. Other viscera and groups of lymph nodes showed no evidence of tumour. Histological examination showed a rather anaplastic epithelial tumour unlike a primary lung carcinoma and of similar appearance to the thigh lesion and the inguinal node metastases.

DISCUSSION

This patient was the first with sweat gland carcinoma of 4753 patients seen in the last 10 years at a combined clinic for skin tumours at Nottingham. Clinically the tumour is grey to violaceous, arising from the dermis, and may be readily mistaken for a secondary carcinoma or a malignant lymphoma. Miller (1967) reviewed 34 published cases along with 5 of his own. Almost all of these 39 cases were in the second half of life. The usual course had been of a slow-growing tumour (duration varying from 3 months to 48 years), frequently recurring (19 cases). In about 75% of cases the primary site was the head or upper limbs, the lower limbs being affected in only 2 cases. One was a poorly differentiated alveolar and papillary carcinoma of the sole, with femoral node metastases 9 months after excision of the primary lesion (Teloh *et al.*, 1957).

Histologically, the degree of differentiation may vary considerably, even within the same tumour. It may be frankly anaplastic or show well-differentiated tubular or cystic papillary structures. Multiple sections of the tumour must be examined to detect the malignant features, as these may be strictly focal (Miller, 1967). An unusual variant of this tumour is the epidermotrophic eccrine carcinoma first reported by Pinkus and Mehregan (1963). The primary tumour on the ankle region was followed by widespread nodular cutaneous metastases, which were partly intraepidermal. The relevance of the intraepidermal spread of adnexal cancer cells to the view that extramammary Paget's disease represents epidermotrophic apocrine adenocarcinoma is discussed by Pinkus and Mehregan (1969).

I wish to thank Dr D. I. McCallum (Nottingham General Hospital) for his permission to publish this case.

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Br. J. Derm. (1972) **86**, 98.

Society Proceedings

ROYAL SOCIETY OF MEDICINE

SECTION OF DERMATOLOGY

President—Dr D. I. WILLIAMS

Thursday, May 20th, 1971

Hendersonula Toruloidea Infection.

—Dr A. KURWA and Dr C. CAMPBELL.

A man aged 51.

History.—At the age of 4 he injured his right big toe nail which has remained dystrophic. He came to England from Jamaica 28 years ago. In his occupation as an engineer his hands get dirty and he needs to wash them very frequently. For 4 years he has noticed swelling of the finger tips and changes in the finger nails.

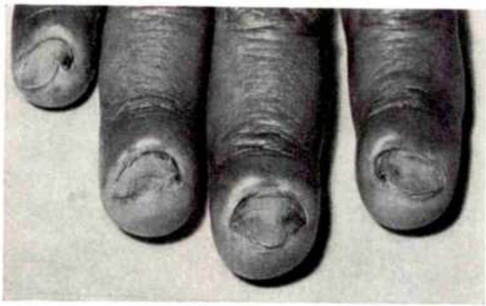

FIG. 1.—Paronychial swelling of most fingers with dystrophy of the nails.

On examination.—Paronychial swelling of most fingers with dystrophy of the nails (Fig. 1). The sides of the nails are darkened and hyperkeratotic. Scaly rash of both insteps with hyperkeratosis of the plantar creases. Right 1st, 2nd and 4th toe nails dystrophic. Left big toe nail discoloured and dystrophic.

Investigations.—Microscopy: potash preparations of the finger nail showed that the infecting fungus was not a dermatophyte. The hyphal walls were olive brown and the arthrospores were thick walled, robust structures. In one sample the young hyphae extending from the brown mycelium were colourless and indistinguishable from those of a dermatophyte. Culture: media used routinely for culture: (i) Sabouraud's glucose agar incorporating cyclohexamide (0·5 mg/ml) and chloramphenicol (0·05 mg/ml). (ii) Malt extract agar, incorporating chloramphenicol (0·05 mg/ml). Inoculated Petri dishes were incubated at 27°C. No isolations occurred on the Sabouraud's agar with cyclohexamide. Growth of *H. toruloidea* on malt extract agar without cyclohexamide was rapid, reaching a colony diameter of 35 mm in 4 days. The aerial growth was mouse-grey, loosely fluffy, 5–10 mm high and exhibited marked growth rings (Fig. 2). The reverse was mottled, black in the centre, becoming colourless at the margin. Microscopically the only significant feature was the abundance of arthrospores formed from the fragmentation of the older hyphae. These were colourless, long oblong (3·5 μ wide $\times$ 11 μ long) to olive-brown, barrel shaped,

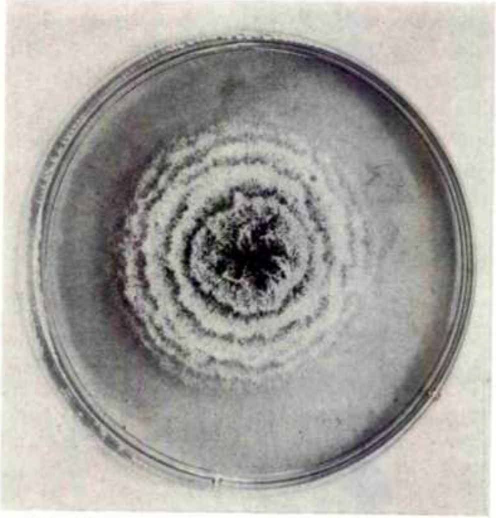

FIG. 2.—Loose fluffy aerial growth on Malt extract agar.

(7·5 μ wide × 5 μ long) often adhering in chains. The strain isolated from the left great toe nail differed from the others in having a much slower growth rate and a higher, denser aerial turf. The fungus was ascribed to the "Torula" stage of *Hendersonula toruloidea* Nattrass. Sensitivity to griseofulvin: the first isolate from the finger nail was grown in the presence of 12 levels of griseofulvin in liquid culture and found to be uninhibited at the highest level tested (100 μg/ml).

Comment.—The genus Hendersonula is a division of the family Sphaeropoidaceae, Order Spheropsidales, Fungi Imperfecti. There are some 10 species, mostly weak parasites on the aerial parts of plants. *H. toruloidea*, first described by Nattrass (1933), characteristically occurs on wood or bark and is widespread in the tropics. There is no record of its occurrence in Britain on these substrates. Gentles and Evans (1970) reported 8 cases of infection of the feet and nails with *H. toruloidea*. Seven of their patients came from the tropics, as did ours. All had infection of the toe cleft and 4 had infection of the toe nails. This is the first reported case of chronic paronychia and finger nail infection with *H. toruloidea*. The sensitivity of our isolate to cyclohexamide and its resistance to griseofulvin agrees with the findings of Gentles and Evans.

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ROYAL SOCIETY OF MEDICINE

SECTION OF DERMATOLOGY

President—Dr D. I. WILLIAMS

Thursday, June 17th, 1971

Herpetiform Pemphigus Foliaceus Responsive to Dapsone.—Dr B. CONNOR.

A man aged 58.

History.—Six years ago mildly pruritic lesions appeared on the face with extensive involvement of the trunk and limbs during the next 6 months. No past or family history of skin disease. Chronic bronchitis and dyspepsia suggestive of a duodenal ulcer had been present for 10 years. Rheumatoid arthritis developed 18 months ago, treated with indomethacin.

On examination.—There were crusted erythematous plaques 2 to 5 cm in diameter disseminated over the face, scalp and trunk (Fig. 1). Initially these lesions closely resembled seborrhoeic eczema. There was no aggregation of the lesions over the extensor surfaces, shoulder, sacrum or scalp as occurs in dermatitis herpetiformis (DH). Older lesions had healed spontaneously leaving pigmented macules. Axillary and inguinal lymphadenopathy, bilateral gynaecomastia and clubbing of the fingers were present. Active rheumatoid arthritis involving the hands, wrist, ankle and knee joints was evident.

Investigations.—Histology: superficial splitting of the epidermis at the level of the stratum granulosum with acantholysis (Fig. 2). Tzanck smear: many acantholytic cells seen. Hb. 13·1 g, W.B.C. 7,900 (74% N; 14% L; 4% E; 8% M), E.S.R. 54 mm/h. Rose Waaler Test positive 1 : 250. Antinuclear Factor, W.R. and V.D.R.L. negative. Immunoglobulins; IgG 2,000, IgA 820, IgM 200 mg/100 ml. X-rays. Hands show rheumatoid erosions and osteoporosis: chest and hilar tomograms normal. Barium meal and gastroscopy: distension and inflammation of the upper gastric mucosa. Gastric and jejunal mucosal biopsies normal. Axillary

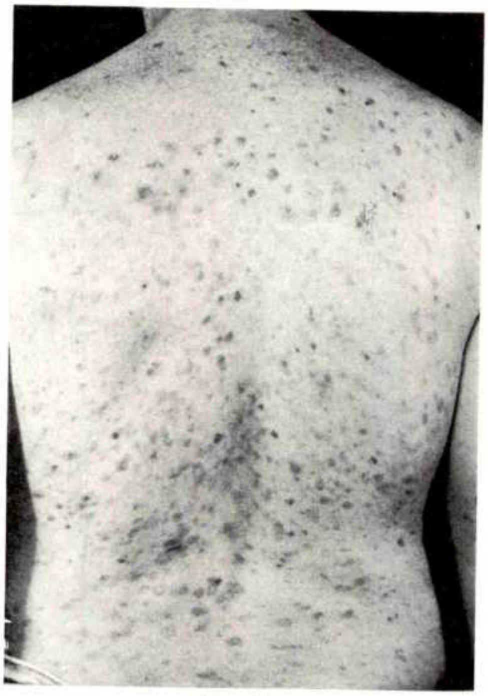

FIG. 1.

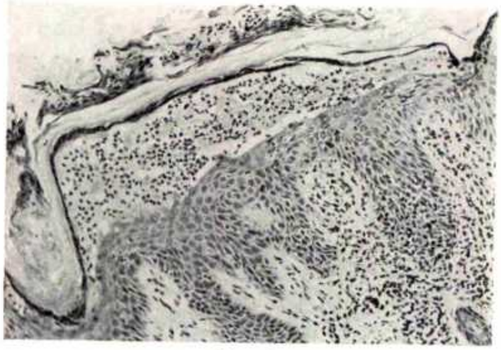

FIG. 2.

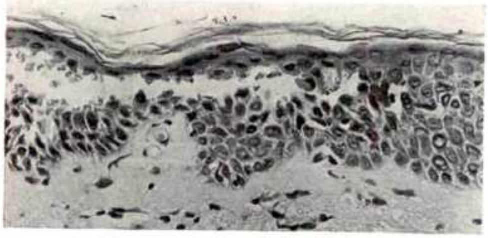

FIG. 3.

lymph node biopsy: reactive hyperplasia only. Indirect immunofluorescent test for serum anti-epithelial antibody positive to titre of 1 : 20 (off dapsone 7 days) (Dr H. Amos, Department of Immunology). Liver function tests, blood urea and electrolytes, serum protein and P.B.I., xylose absorption and glucose tolerance tests normal. When an area of normal appearing skin was irradiated with u.v. light from a monochromator a normal response was obtained to a minimal erythema dose using wavelengths of 300 and 307·5 nm. A biopsy taken from this site 24 h later revealed superficial clefting of the epidermis with acantholysis (Fig. 3). The amount of irradiation given was approximately 660 mW/cm².

Progress.—Fluocinolone acetonide 0·2% ointment partially controlled the disease for 3 years. In July 1968 the lesions resembled those seen in DH with small groups of erythematous plaques, some studded with tense vesicles. A trial of dapsone 100 mg/day was given with relief of pruritus in 48 h and marked clearing of the lesions after 7 d. Six weeks later dapsone was omitted with recurrence of pemphigus 5 d later. The histology of these " withdrawal " lesions showed superficial splitting of the epidermis with acantholysis (Fig. 4). Two weeks later Sulphapyridine 1·5 g daily was administered for 14 d, the response being inferior to that obtained with dapsone. Thereafter dapsone, 100–150 mg/d has controlled the pemphigus with no complications for over 3 years. Recurrence still occurs if dapsone is omitted. Systemic steroid therapy was not given because of a possible peptic ulcer.

Comment.—This patient is very similar to the 2 patients reported by Winkelmann and Roth (1960) who also responded to dapsone and sulphamethoxypyridazine. However, 2 other patients with a similar clinical picture failed to respond to dapsone and needed systemic corticosteroid therapy (Sneddon and Church, 1967). In these 5 patients, although the individual lesions resembled those of DH, they failed to show the characteristic localization which occurs in DH. In addition, there was no evidence of malabsorption and the

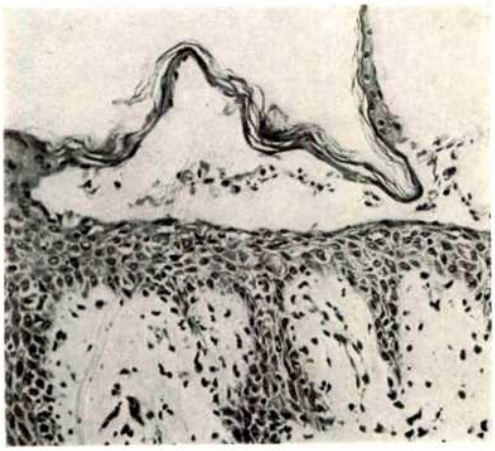

FIG. 4.

jejunal mucosa was normal, whereas in DH 70% of patients have an abnormal jejunal mucosa (Marks *et al.*, 1968). The histology confirmed pemphigus foliaceus on 4 separate occasions, there being no features of DH. Cram and Winkelmann (1965) noted similar responses to u.v.r. in 7 patients.

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The following cases will be published in full in the *Proc. R. Soc. Med.*

Malignant Melanomata in Lymphoedematous Arm Following Radical Mastectomy for Breast Carcinoma (An Extension of the Syndrome of Stewart and Treves).—Dr I. SARKANY.

Scleredema of Buschke.—Dr J. S. PEGUM.

Mycosis Fungoides with Dermatomyositis.—Dr B. CONNOR.

Correspondence

Br. J. Derm. (1972) **86**, 102.

Psoriasis and Hydroxyurea

SIR,—In several publications (Leavell and Yarbro, 1970; Stein *et al.*, 1971; Rosten, 1971) concerning the treatment of psoriasis with hydroxyurea, the authors noted the good haematological tolerance of this drug. The only reported anomaly is a moderate leukopenia which regresses rapidly after the treatment is stopped.

We have treated 5 patients with psoriasis with hydroxyurea, in a daily dose of 0·5 g to 1·50 g. In 4 cases, we have found, after 3 or 4 months, an important macrocytosis with diminution of the erythrocyte count (1·5 millions on an average) but no change in the haemoglobin level.

		Before hydroxyurea	After hydroxyurea	Length of treatment Total dose
Case 1	M.C.V.	102 μ^3	108 μ^3	110 days
	R.B.C.	4·4 M	2·90 M	163 g
Case 2	M.C.V.	102 μ^3	144 μ^3	150 days
	R.B.C.	4·7 M	3·3 M	125 g
Case 3	M.C.V.	93 μ^3	122 μ^3	145 days
	R.B.C.	4·75 M	3·39 M	150 g
Case 4	M.C.V.	95 μ^3	129 μ^3	115 days
	R.B.C.	4·45 M	3·2 M	83 g
Case 5	M.C.V.	93 μ^3	92 μ^3	28 days
	R.B.C.	4·17 M	4·6 M	42 g

We have measured the serum folate level in 3 of the preceeding cases and have found one normal figure (6·2 μg/ml, case 2), one distinctly low level (2·15 μg/ml, case 1) and one impossible to interpret (absorption of folic acid the day before).

The oral administration of folic acid (pteroyl-glutamic acid) to those last 2 patients does not appear to have hastened the recovery of the red cell anomalies, after the cessation of hydroxyurea.

Macrocytosis with a normal serum folate level has already been reported. It usually occurs earlier. The mechanism remains debatable (Bergsabel *et al.*, 1964) and requires more extensive study.

We wish mainly to stress the great frequency with which we have observed this phenomenon in the course of treatment of psoriasis with hydroxyurea.

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"Lymphocyte Transformation" (*Br. J. Derm.*, 1971, **85**, 300)

SIR,—We referred to lymphocyte transformation rather than thymidine uptake ratio because we have found that these 2 tests measure slightly different parameters. The "lymphocyte transformation" as measured by counting transformed cells in a stained film was found in our hands to be more informative than the thymidine uptake ratio. In a stained film "toxic" changes in the cells can be detected simultaneously with "blast cell transformation" and the dose of the stimulating agent can then be varied In the thymidine uptake ratio this may show no reaction because (1) The dose of the stimulating agent was too high, (2) too low, (3) too toxic. No information to which of the 3 is given while a clue can be obtained by the much more tedious methods of counting and looking at the cells—hence the terminology.

Messrs Hutchinson, Macleod and Raffle appear to be worried that we did not take a sufficient number of readings. The sentence "tests were carried out in duplicate as far as was possible, using both the direct method and . . . that of labelling the cultures . . ." means that we used both methods where we had enough blood,—perhaps "in parallel" would have been a better expression—it does certainly not mean that when we used the thymidine uptake method we only used one aliquot: all our tests, routinely, are done on 3 aliquots for each strength of drug used, and in fact when we did the test in "duplicate" we had 3 aliquots for each concentration of drug for the thymidine uptake and 2 for the counting method. The distintegrations per min given in Fig. 2 were the *average* readings of each of these 3 aliquots, and where one reading varied by more than 20% from the others, all 3 were discarded.

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Books

Common Skin Diseases. HOWARD T. BEHRMAN, THEODORE A. LABOW and JACK H. ROZEN (1971) New York: Grune & Stratton. Pp. 189, 260 coloured illustrations. Price £6.80.

This book, slim in relation to its content, purports to be a simple and concise manual of dermatology for the student and the general practitioner and, in this second edition, "to steer them through the uncharted wilds of newer therapeutic agents, while alerting them to their availability". The profuse and completely relevant colour illustrations, which make it costly but unique among books of its kind, are matched by the succinct aetiological preambles and basic clinical features of the carefully selected dermatoses. The failure of international drug firms to give the same name to the same product in Cincinnati and Cirencester lowers this high standard in the therapy paragraphs and terminal tabbed formulary section, as does the authors' frequent tendency to advocate investigations and treatments only to dismiss them then as valueless. The recommendation, without comment, of hazardous or sensitizing drugs and local applications, and of x-radiation in some undeserving conditions, together with the inclusion of hoary, if money-spinning, anachronisms such as staphylococcal toxoid for folliculitis, Thorium X for naevus flammeus, or repeated vaccinial vaccination for recurrent herpes simplex seem further to detract from the book's suitability for those with "limited access to a consultant". In summary, the authors present a beautifully and profusely illustrated book, the price of which would suit the library of the Medical School, Health Centre or Group Practice but not, one regrets, the pocket of the student for rapid cover or revision of a subject still inadequately taught.

IAN W. CALDWELL

Br. J. Derm. (1972) **86**, 104

Quarterly Review

CONTACT DERMATITIS XVII

Reactions to Contact Allergens given Orally or Systemically

It is known that ingestion of a contact allergen in a sensitized person can cause dermatitis. How often this happens or the part this mechanism plays in the chronicity of eczema is unknown.

Medicaments

The serious effects that may occur were emphasized during the Second World War by reports from troops who were given courses of oral sulphonamides having been previously sensitized by their topical use. Twelve such cases were described by Park (1943) who found that the severity of the eruption varied with the sensitivity of the subject and the size of the dose of sulphonamide given. In the same patient a large amount caused a severe reaction and a small dose a mild effect. In 60% of cases the allergy was to one sulphonamide only, whereas in 40% it could be elicited by several (Park, 1944). Sulphanilamide in powder or petroleum jelly was the most frequently used topical application and caused the majority of reactions (Grant Peterkin, 1945). A surprizing but apparently consistent feature of many of these patients was their negative response to patch testing with sulphonamides (Park, 1943; Grant Peterkin, 1945). Sulzberger *et al.* (1947) recorded that of all their patients only 31% gave a positive patch test but in those with severe reactions all were positive.

When streptomycin was introduced for the treatment of tuberculosis the risk of contact dermatitis among the nursing staff was not realized and some were sensitized. Eighteen nurses with this contact dermatitis were described by Wilson (1958) and 6 were desensitized by subcutaneous injections of streptomycin. During this period of desensitizing injections 5 nurses developed eczema and 2 urticaria. On completion of the course patch tests which had been positive with 0·1% aqueous solution of streptomycin were now negative to 20%.

Nurses who handle chlorpromazine are similarly at risk and desensitization has been achieved by giving the drug orally (Morris-Owen, 1963). The patch test was converted from being positive at 0·05% to negative at 0·5% solution. The main reaction during the desensitizing procedure was oedema and irritation of the eyelids.

A dramatic account of repeated eruptions in a neomycin and bacitracin sensitive patient, due to its inadvertent use was described by Pirila (1960). The patient was sensitized to neomycin and bacitracin by its topical use on an amputation stump. Two years afterwards he developed a severe stomatitis from throat tablets containing bacitracin. Many years later a paste containing both antibiotics was used to fill an infected root canal. Within 1·5 hr the patient's palms felt itchy, within 4 hr he felt unwell and the same day his face became red and oedematous. He was generally itchy, the irritation being pronounced in certain sites including the amputation stump. There was also swelling of the mucosa of the mouth and pharynx. Once the patient was well 1·3 g of neomycin was given orally during 24 hr. Within 6 hr the patient developed diarrhoea and by the following day had a widespread dermatitis, which was severe enough to necessitate oral steroids.

Deliberate administration of neomycin orally to 12 neomycin sensitive patients was also described by Ekelund and Moller (1969). Of this group 10 had some reaction, in 6 the area of the previous patch test flared, and in 5 there was a relapse at the site of their original dermatitis. Other effects were vesiculation of the palms, papules on the back and groins, generalized dermatitis and itching and nausea. Ekelund and Moller also gave quinolines to 9 patients sensitive to these antibiotics. In 6 the result was positive, the commonest reaction being a flare of the original dermatitis. Itchy papules either scattered or localized also occurred, but in none was the patch test site reactivated. Among this group of 16 positive reactors 4 were described as having a toxicoderma-like rash. A similar case in which diodoquin caused a severe exacerbation of eczema was reported by Leifer and Steiner (1957).

In contrast to these findings are those of Maibach (personal communication) in subjects experimentally sensitized by the Draize test to neomycin. After 18 months they still reacted on patch testing to 20% neomycin, and 6 were given 1 g of neomycin daily for 5 days. Neither dermatitis nor a flare of the patch test occurred.

Penicillin in milk was found to be the cause of attacks of eczema in 2 patients, who were both sensitive to penicillin on patch testing (Vickers *et al.*, 1958).

Vitamin B_1 (thiamine) given orally has also been described as causing a relapse and exacerbation of eczema in a girl who had become sensitized to this vitamin through handling it at work (Hjorth, 1958).

Paraphenylenediamine—Group

The possibility that azo dyes in food might cause dermatitis in PPD positive subjects has been investigated (Baer and Leider, 1949). Twenty subjects were fed 15–210 mg of one of the food azo dyes. In 7 there was a visible flare of their eruption but the response was not always reproducible and a similar response occurred in 2 of a group of 20 controls. The authors said it was difficult to draw definite conclusions from these results and pointed out that a pound of butter or margarine contained only 17 mg of azo dye. An interesting study of the injection and ingestion of allergens of the para-group in sensitized individuals was reported by Sidi and Dobkevitch-Morrill (1951). Giving 0·5 g of sulphonamide orally to patients sensitized topically caused fever, syncope, delirium and erythroderma. Similarly when procaine was given by mouth to these subjects a reaction resulted in nearly half, causing eczema in previously affected areas, a spread of the eczema, diarrhoea and fever.

Procaine sensitive patients were given sulphonamides orally and some developed a photo-dermatitis. A paraphenylenediamine sensitive patient had no response to oral procaine and although a patch test to sulphonamide was negative he reacted to it when given orally. Similarly a patient sensitive to stocking dyes after being given sulphonamides became febrile with a spreading eczema. Three PPD-sensitive subjects had 1 ml of a 2% solution of procaine injected, in 2 there was no response but the third patient collapsed with fever, facial oedema and a vesicular eczema of the dorsum of the hand. A slough formed at the injection site.

Mayer (1949) postulated that PPD breaks down to a quinoneimine structure to become antigenic. Several antihistamines have the potentiality to form these groups and on this basis Mackie and Mackie (1964) postulated that antihistamines prolong dermatitis due to hair dye and reported that this had been their clinical experience.

Thiuram Chemicals

A patient sensitized to dipentamethylene thiuram disulphide from a rubber contraceptive developed a violent reaction and a widespread eczema when given antabuse (tetraethylthiuram disulphide) for the treatment of his alcoholism (Wilson, 1962). A similar patient was described by Pirila (1957) the reaction occurred within 4 hr, and a thiuram patch test which had faded was reactivated.

Flavours

Hjorth (1961) reported 2 children who were sensitive to balsam of peru and orange peel and whose eczema flared after eating fruits and ices. He has also described (1971) a man with severe hand eczema for 10 years who, because he reacted to balsam of peru on patch testing, was told to avoid orange peel. To test the validity of this advice the patient ate a jar of orange marmalade and in 12 hr had his worst attack of hand eczema. He then avoided " perfumes, cola drinks, vermouth, throat tablets, cinnamon, and other items " and his hand eczema cleared. A similar woman with hand eczema due to contact and ingestion of cinnamon was described by Leifer (1951). Eating vanilla sugar has also caused a flare of eczema in a patient sensitive to balsam of peru (Pirila, 1970) and it is possible that garlic may also have this effect (Burks, 1954).

Chromium

Five patients who were allergic to chromium were given 50 μg of potassium dichromate orally by Fregert (1965). Wthin 2 hr they had all developed severe vesiculation of the palms and one patient had a generalized eruption. In another study (Schleiff, 1968) 20 chromium sensitive patients were given 1–10 mg of dichromate by mouth and in nearly all there was a flare of previous eczematous sites and in some reactivation of positive patch tests. This same effect was reported by Sidi and Melki (1954). The intake of chromium in food is about 30–100 mg daily (Schroeder *et al.*, 1962). Hexavalent but not trivalent chromium is well absorbed from the gut but as the acidity of the gastric juice maintains the trivalent form it is likely that in a normal person little chromium is absorbed (Donaldson and Barreras, 1966). The investigations of Fregert (1965) and Schleiff (1968) mean that even the trace amounts absorbed from the gut can cause eczema in a sensitized subject.

An acute flare of a vesicular eczema of the palms occurred in a chromate sensitive man who inhaled fumes, containing chromium from an acetylene welding operation (Shelley, 1964).

Other Allergens

In 1958, Kligman gave 20 mg of pentadecylcatechol intramuscularly to 11 patients with poison ivy dermatitis to disprove the claim that giving the antigen parenterally would cure the eruption. Two patients were severely ill with malaise and leucocytosis, one had meningismus and one had urticaria, both had a generalized exanthem and severe inflammation at the site of injection. In the other patients the main reactions were urticaria, exanthem and also severe inflammation at the injection site. Ethylenediamine has been reported as causing eczema after ingestion (Provost and Jillson, 1967).

Mechanism

Striking features of many of these case reports are the rapid onset, within hours, of the effect of the oral or systemic antigen and when the effect is severe, the systemic upset experienced by the patient. Such rapidity of response suggests that an immediate rather than a delayed type hypersensitivity may be partly or entirely responsible.

The mechanism whereby intravenous dichromate could reactivate previously positive patch tests in dichromate sensitive guinea-pigs was investigated by Polak and Turk (1968). They found that the response began within 2–4 hr of the injection and the inflammation was caused by a perivascular polymorphonuclear infiltrate. It was blocked by giving anti-granulocytic serum, which does not interfere with delayed type hypersensitivity reactions and was unaffected by antilymph node permeability factor, which suppresses delayed type hypersensitivity. They concluded that this effect was possibly mediated by humoral antibody rather than immunologically competent cells. The same may be true in the human subject.

Comment

These reports prove only that in some subjects the ingestion or systemic administration of an allergen can cause both eczema and systemic effects, equally in other patients no reaction occurrs. The importance of this mechanism or its frequency have never been assessed.

St John's Hospital
London W.C.2.

Etain Cronin

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News and Notices

BAD Summer Meeting, London, July 6th, 7th and 8th. Request for Papers

Papers for this Meeting are requested. They should be submitted by March 1st, 1972, in the form of a summary of approximately 200 words. The summary should be as precise as possible, avoiding vague statements (e.g. the results will be discussed). The summary should be sent to the Hon. Secretary, Dr J. S. Pegum, 53 Philpot Street, London, E.1.

Dowling Fellowship 1971

The Dowling Fellowship for 1971 has been awarded to Dr Martin Black. He will spend a year in the Department of Dr William Epstein at the University of California School of Medicine, San Francisco.

Alfred Marchionini Foundation

After the conclusion of the 29th Congress of the German Dermatological Society the Council of the Alfred Marchionini Foundation met at the Hotel Kempinski, Berlin, on October 3rd, 1971.

In the presence of the founder, Kurt Herrman, and the honorary members, Professor Jadassohn (Geneva), Longhin (Bucharest) and Monacelli (Rome), the Council chose the recipients of the Alfred Marchionini Gold Medal and the Alfred Marchionini Prize. All the members of the Council attended the meeting: Professor Götz, Chairman (Essen) and Professors Baer (New York), Braun-Falco (Munich), Kimmig (Hamburg) and Niebauer (Innsbruck), Dr Rook (Cambridge) and Professor Sagher (Jerusalem).

Royal College of Physicians, Edinburgh

The following dermatologists were recently elected to the Fellowship of the Royal College of Physicians, Edinburgh:

Edmund James Raffle, M.B., Liverpool.
George Kenneth Hargreaves, M.B., Edinburgh.
Hugh Woolf Chadfield, M.R.C.S. Eng., L.R.C.P., London.
Patrick James Hare, M.D., London, F.R.C.P., London.

New Journal

The first issue of the *Journal of Immunological Methods* was published in September 1971. It is intended that the journal shall eventually appear monthly, but it will at first be published quarterly. The editors are F. Bocek and J. R. Battisto of the Albert Einstein College of Medicine, New York and E. J. Holborow of the M.R.C. Unit, Taplow. The journal is published by the North-Holland Publishing Company, P.O. Box 3489, Amsterdam, and the annual subscription is $25.

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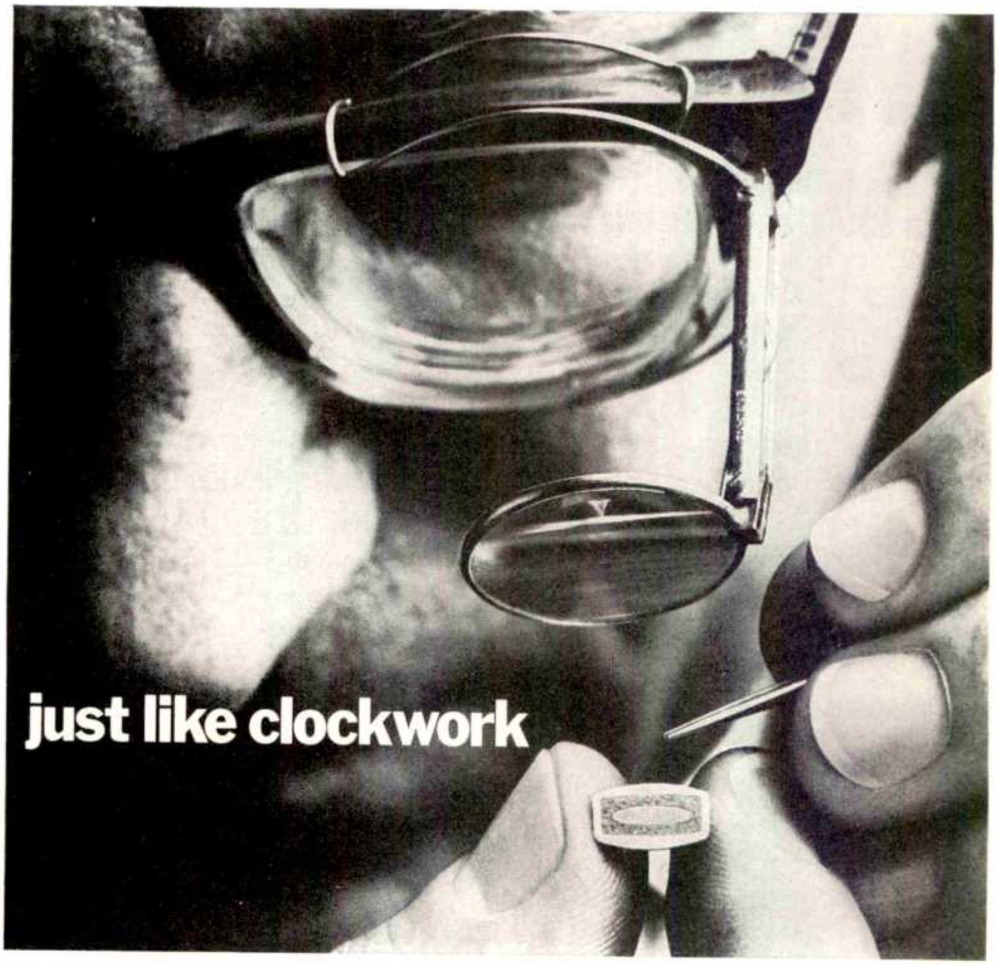

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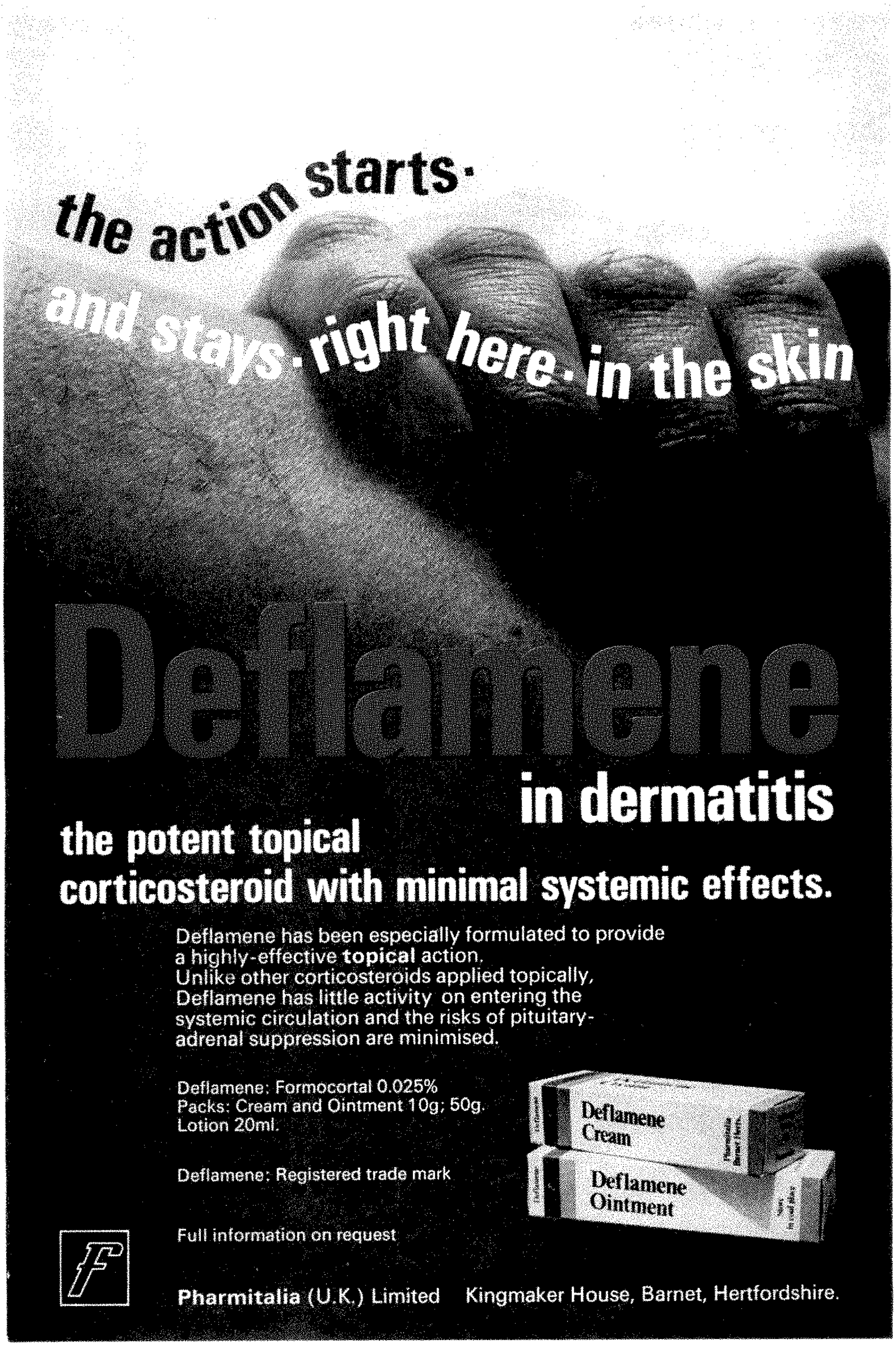

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CONTENTS (*continued*)

Case Reports

Acrokeratosis Verruciformis of Hopf with Steatocystoma Multiplex and Hypertrophic Lichen Planus
JULIAN VERBOV 91
Eccrine Sweat Gland Carcinoma with Metastases
VINAY K. DAVE 95

Society Proceedings

Royal Society of Medicine, May 20th and June 17th 1971 . . . 98

Correspondence

Psoriasis and Hydroxyurea
R. TOURAINE, J. REVUZ and M. TULLIEZ 102
" Lymphocyte Transformation "
SUZANNE ALEXANDER 103

Books

Common Skin Diseases (Behrman, Labow and Rozen) 103

Quarterly Review

Contact Dermatitis XVII. Reactions to Contact Allergens given Orally or Systemically
ETAIN CRONIN 104

News and Notices 108

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

A Survey of Ichthyosis Vulgaris in Israel
L. Zlotkowski and A. Feinstein 1
Disseminated Elastosis Perforans Serpiginosa in Four Mongoloids. Recognition of Residual Changes
James E. Rasmussen 9
Epidermal "Turnover Time,"—a Re-examination
Kenneth M. Halprin 14
Histochemical Applications of Skin Surface Biopsy
R. Marks 20
Ultrastructure of Adult Epidermal Cells in Monolayer Culture
Hilary Constable 27
Experimental Zosteriform Herpes Simplex Virus Infection in Mouse Skin
T. W. E. Robinson and J. R. Dover 40
The Control of Glycogen Utilization in Skin; Phosphorylase *B* Kinase and Phosphorylase *A* Phosphatase
P. D. Mier and A. H. M. Sutorius 49

Pharmacology and Treatment

Treatment of Vitiligo with Oral 4,5′,8-Trimethylpsoralen (Trisoralen)
S. S. Bleehen 54
The Effect of Prolonged Topical Application of an Anticholinergic Agent on the Sebaceous Glands
M. Cartlidge, J. L. Burton and S. Shuster 61
Effect of Topical Tetraynoic Acid on Sebum Excretion
T. S. Haroon, J. Hall and A. Haroon 64
The Effect of Topical Tetraynoic Acid on the Seborrhoea of Acne
J. L. Burton and Sam Shuster 66
A Study of the Effect of Prednisone and an Antihistamine on Patch Test Reactions
E. Feuerman and A. Levy. 68
Treatment of Idiopathic Hyperhidrosis with Iontophoresis of Tap Water and Poldine Methosulphate
Katherine Grice, H. Sattar and Harvey Baker 72
Treatment of Psoriatic Nail Dystrophy
E. Abell 79
Penetration and Dose of Injections with the Porton Jet Injector
P. Gaylarde, A. L. Macmillan and I. Sarkany 83

Comment

Antimetabolites and Psoriasis
Peter C. H. Newbold 87

(*Continued on* p. iii *of cover*)

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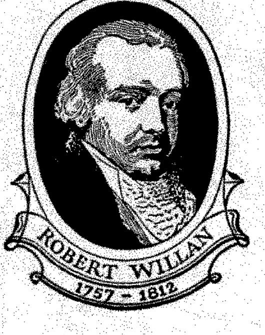

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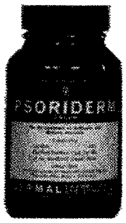

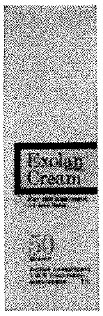

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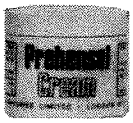

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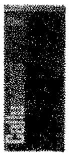

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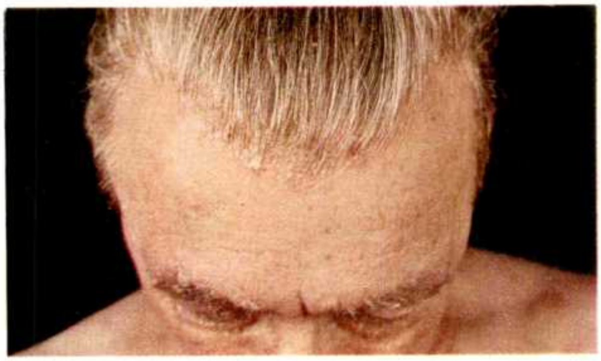

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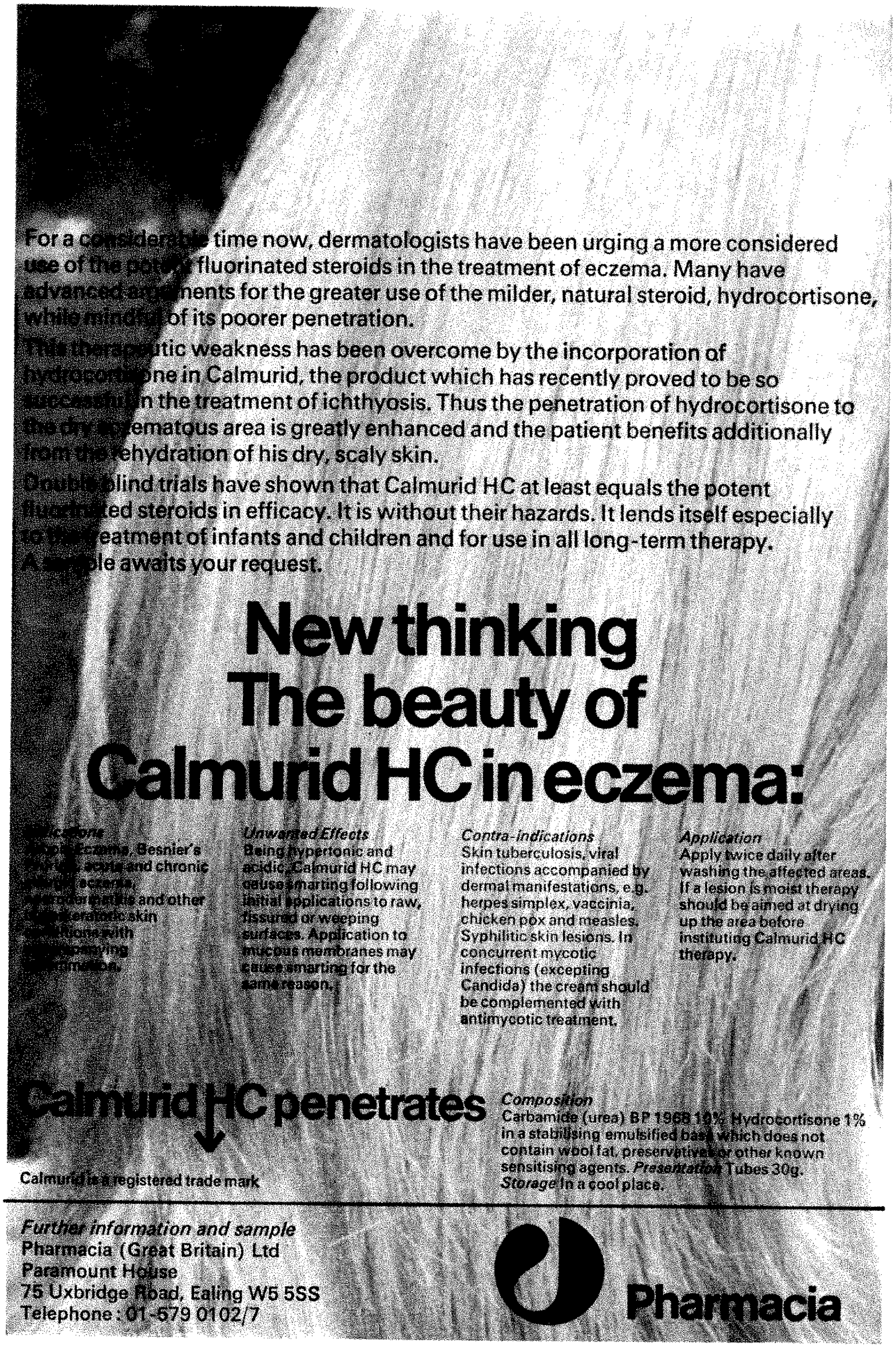

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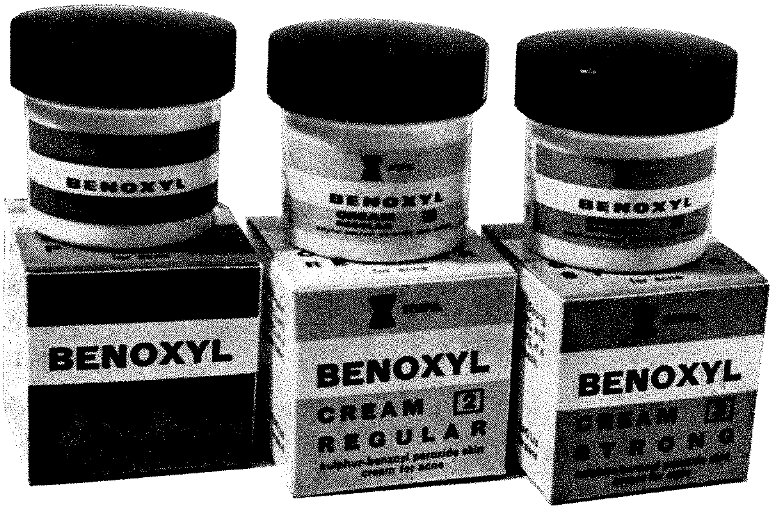

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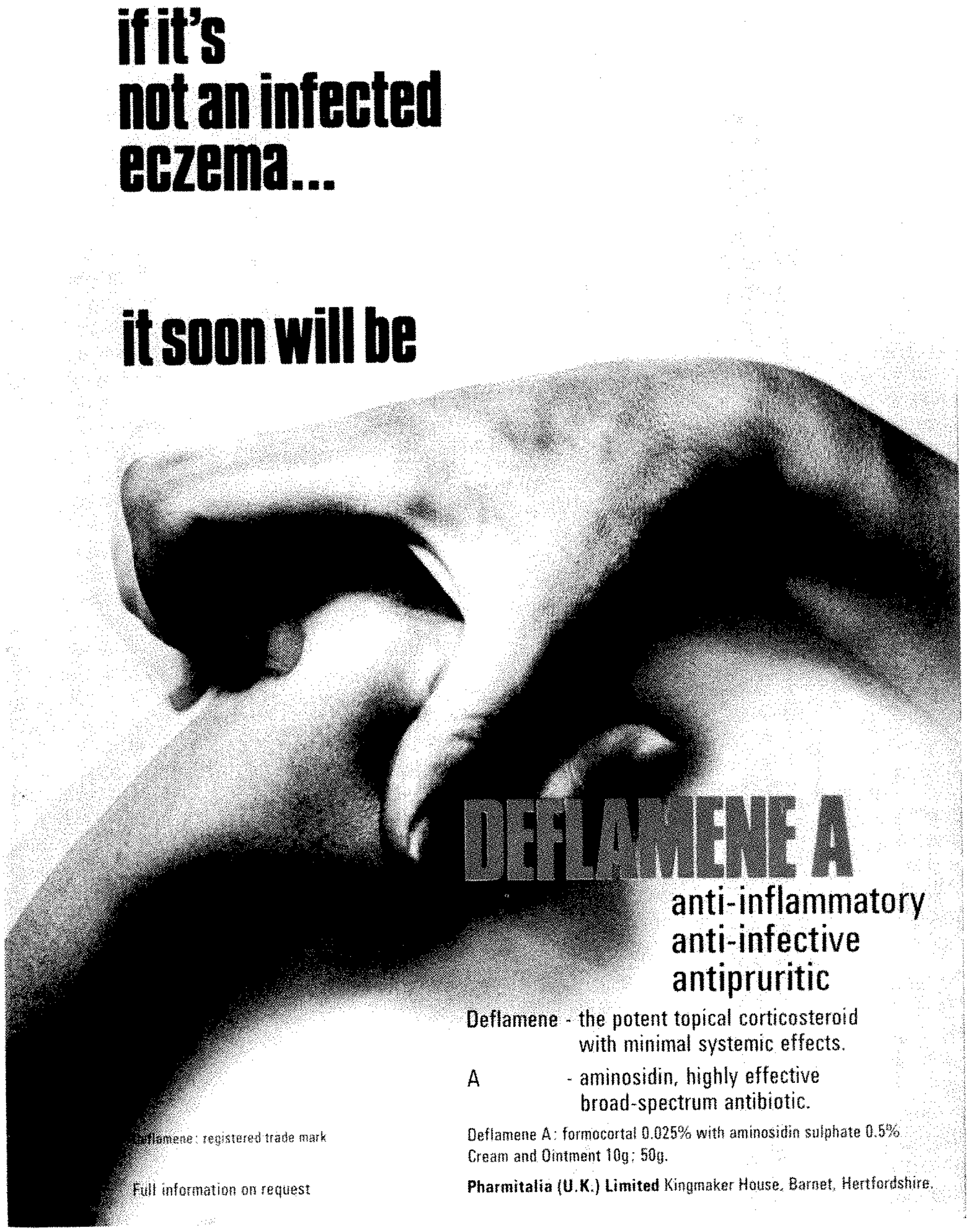

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Clinical and Laboratory Investigations

Br. J. Derm. (1972) **86**, 111.

Department of Microbiology, School of Medicine, State University of New York, Buffalo NY 14214

FACTORS CONTRIBUTING TO OCCASIONAL FAILURES IN THE LABORATORY DIAGNOSIS OF BULLOUS PEMPHIGOID BY INDIRECT IMMUNOFLUORESCENCE*

TADEUSZ P. CHORZELSKI† AND ERNST H. BEUTNER

Summary.—The basement membrane antibodies in 3 sera of patients with bullous pemphigoid were studied by means of indirect immunofluorescent staining chessboard titrations on monkey, rabbit and guinea-pig lip tissue. Comparisons of the results of these studies with those obtained in previous studies of this type with 2 other sera, revealed wide variations in the spectra of specificities of the bullous pemphigoid antibodies. Monkey oral or oesophogeal mucosa appears to be the substrate of choice for routine tests for these antibodies, although this must be regarded as an approximation of the " ideal antigen ".

The implication of these findings are discussed both from the practical and theoretical points of view.

FINDING the basement membrane antibodies of bullous pemphigoid is essentially diagnostic for the disease (Jordon *et al.*, 1967; Jablonska, 1970; Cormane and Szabo, 1970; Sams, 1970; Peck *et al.*, 1968). These antibodies can be detected in only about 70% of the cases, while the *in vivo* binding can be demonstrated in virtually all cases with active lesions (Jordon *et al.*, 1971; Sams, 1970). The studies set forth in this report reveal that some of the failures to detect circulating bullous pemphigoid antibodies are due to the use of non-reactive antigens.

METHODS

The sera selected for this study were from clinically typical cases of bullous pemphigoid in which the diagnosis was confirmed by the established histological and immunopathological procedures. The latter included both the direct immunofluorescent (IF) study of skin biopsies and indirect IF tests of sera with standard procedures using a conjugate with a molar F/P = 3·9 at a dilution containing 100 μg/ml. Some of the sera used in this study were selected on the basis of their failure to react with guinea-pig or rabbit tissue under these conditions.

For the purposes of the experimental studies here reported IF tests were performed with a conjugate with somewhat different characteristics. Specifically the anti whole human IgG conjugate (with antibodies to light chain) had the following characteristics: F = 46 μg/ml; P = 11 μg/ml; Ab = 4·3 μg/ml; F/P = 4·2 μg/ml = 1·7 m/m; Ab/F = 93 μg/mg = 0·23 m/m.

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† From the Warsaw Medical Academy Warsaw Poland.

Requests for reprints should be addressed to Dr Beutner.

The latter obviously meets the specification previously recommended for minimal Ab/F ratio for tests for skin antibodies, i.e. at least 10 mg/mg or 0·025 molar. However, the F/P ratio of the conjugate employed for this study was significantly lower than the recommended F/P = 3 to 5 m/m. The reason for the selection of a conjugate with an

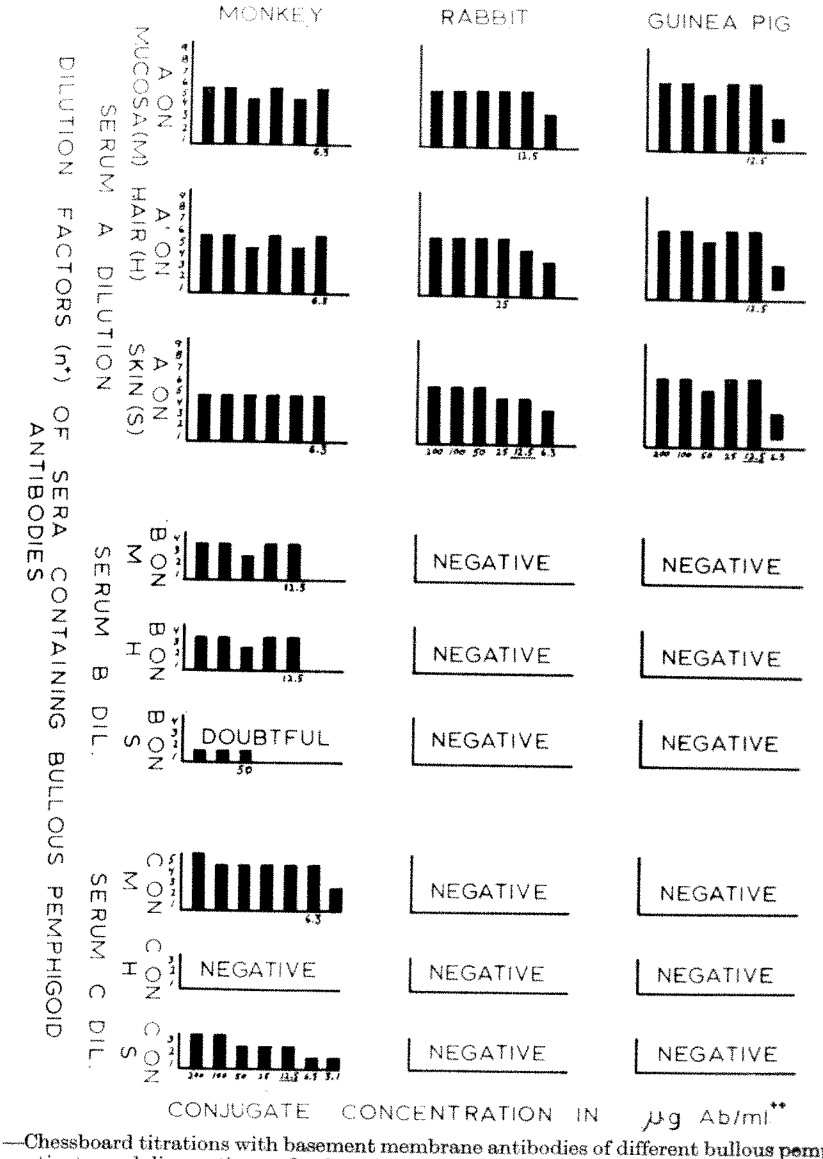
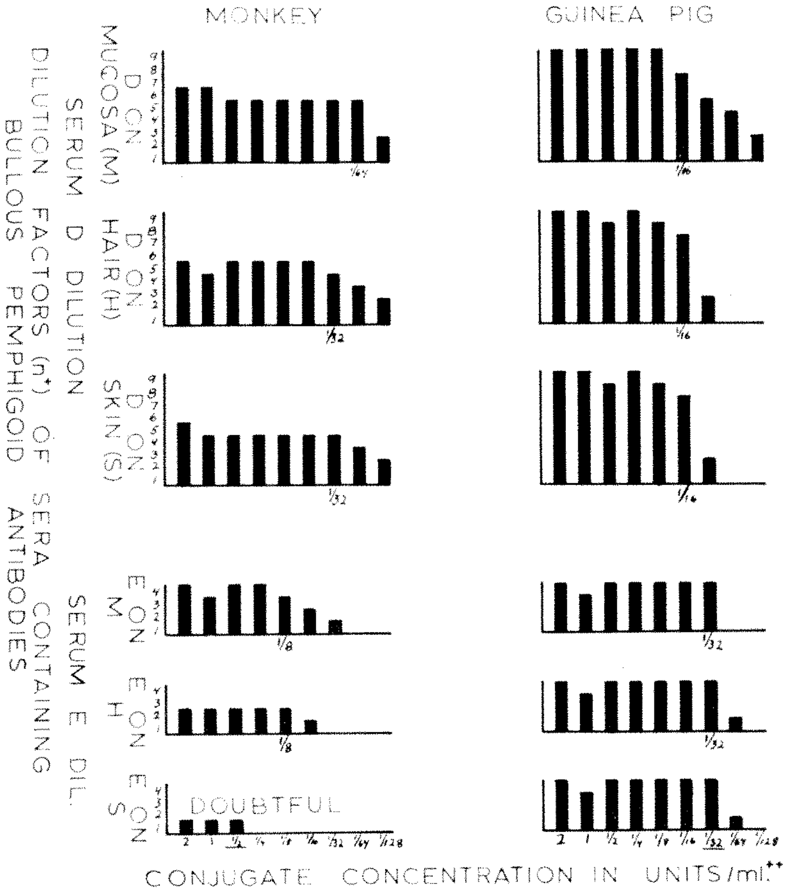


FIG. 1.—Chessboard titrations with basement membrane antibodies of different bullous pemphigoid patients and lip sections of selected species. The black bars depict demonstrable immunofluorescent staining of the basement membrane of the oral mucosa (M), the hair sheath epithelium (H) and the skin (S) at the given dilutions of the sera A to E and the indicated conjugate concentrations. These chessboard titrations on sections of monkey, rabbit and guinea-pig lip reveal that 4 of the 5 sera, i.e. sera from bullous pemphigoid cases A, B, C and E, have clearly distinguishable specificities, as is evident from the divergent reaction patterns on the different structures in the tissues of the species examined.

F/P = 1·7 is that this served to reduce the volume of testing, since titres are lower with such a conjugate than with the standard F/P ratio range of 3·2 to 4·5. The dilutions of this conjugate were adjusted to known antibody protein levels as indicated.

RESULTS

Chessboard titrations of 3 selected sera of patients with clinically typical bullous pemphigoid were carried out on sections of lip from monkey, rabbit and guinea-pig.



[*Foot Notes for Figure* 1.]

* n, the dilution factor of the patient sera is the exponent in the expression: dilution = $1/10 \times 2^n$.
Thus, where n = 0, the dilution = 1/10,
where n = 1, the dilution = 1/20; etc.

** The 2 modes of expression of concentration of the fluorescein-labelled anti IgG conjugates (with H and L chain antibodies) which are employed in Fig. 1 are as follows:

(a) For sera A to C
μg Ab/ml = μg/ml of antibody protein (3·1–200).

(b) For sera D and E
Units/ml = titre of antibody in a standard gel precipitation titration (1/128–2). One unit is roughly equivalent to 200 μg Ab/ml.

These sera designated as A, B and C in Fig. 1 had differing spectra of specificities for the basement membrane of mucosa, hair follicles and skin. Fig. 1 also summarizes the results of chessboard titrations of 2 additional sera designated D and E, which had been reported previously (Wick and Beutner, 1969) since they had led to somewhat different conclusions.

Table I summarizes the results of the same chessboard titrations of the 5 sera in terms of the plateau titres of the bullous pemphigoid antibodies and the plateau endpoint of the conjugate in μg/ml.

TABLE I.—*Summary of Plateau Titres of 5 Sera Containing Bullous Pemphigoid Antibodies and the Plateau Endpoints of Conjugates Used in the Chessboard Titrations*

	Tissue								
	Monkey			Rabbit			Guinea-pig		
Sera	Mucosa	Hair	Skin	Mucosa	Hair	Skin	Mucosa	Hair	Skin
	Plateau titres of sera								
A. (Nev.)	80–160	80–160	80	160	160	160	160–320	160–320	160–320
B. (Gra)	20–40	20–40	10 doubtful	neg.	neg.	neg.	neg.	neg.	neg.
C. (4461)	80–160	neg.	40–80	neg.	neg.	neg.	neg.	neg.	neg.
D. (Sim)	320–640	160–320	160–320	ND*	ND	ND	5120	2560–5120	2560–5120
E. (Hen)	80–160	40	10 doubtful	ND	ND	ND	80–160	80–160	80–160
	Plateau end-points of conjugate in μg Ab/ml								
A. (Nev.)	6·3	6·3	6·3	12·5	25	12·5	12·5	12·5	12·5
B. (Gra)	12·5	12·5	50	neg.*	neg.	neg.	neg.	neg.	neg.
C. (4461)	6·3	neg.*	12·5	neg.	neg.	neg.	neg.	neg.	neg.
D. (Sim)	est.† 6·3	est. 12·5	est. 12·5	ND	ND	ND	est. 12·5	est. 12·5	est. 12·5
E. (Hen)	est. 25	est. 25	est. 100	ND	ND	ND	est. 6·3	est. 12·5	est. 12·5

* ND—not done; "neg." for plateau end-point means negative or greater than 200 μg Ab/ml.

† Since the preceding studies employed conjugates assayed for unitage, only the following conversion table was used as a basis for estimating μg antibody protein (μg Ab/ml). This has a reliability of ± one doubling dilution e.g. 1 unit—100 to 400 μg Ab/ml.

1 unit est.	200
1/2 unit est.	100
1/4 unit est.	50
1/8 unit est.	25
1/16 unit est.	12·5
1/32 unit est.	6·3
1/64 unit est.	3·1

As indicated in Fig. 1, serum A yields reactions with basement membrane antigens of all 3 species tested. Only minor differences appeared in the plateau titres of the serum and the plateau end-points of the conjugate. Specifically the conjugate dilution at the plateau end-point tended to be somewhat higher (6·3 μg Ab/ml) on monkey tissue than on rabbit and guinea-pig, while the plateau titres of serum A were somewhat lower on monkey than on guinea-pig.

On the other hand, sera B and C reacted with monkey tissue, but were completely negative on rabbit and guinea-pig tissue at all concentrations of conjugate tested. Thus, in contrast to serum A, sera B and C appeared to be specific for primate basement membranes within the limits of the tissues tested. Differences in the specificity for different structures within the same species are evident from the comparisons of sera B and C. While both sera had significant titres on monkey mucosa, serum C gave negative reactions with hair follicle basement membranes but significant titres on epidermis. Conversely, serum B had significant titres on hair at the usual conjugate dilutions (used in routine tests) but gave only weak reactions at high concentrations of the conjugate (50 μg Ab/ml or more) on the epidermis. The observed titres of 10 of serum B on epidermis constitute a " doubtful " positive.

Previously published chessboard titrations are included in Fig. 1 and Table I. Interestingly, the sera D and E, which had been used in the previous studies, yielded uniformly strong positive reactions on all 3 types of basement membranes of the guinea-pig lip. Yet serum E (like serum B in the present series) gave doubtful reactions with the epidermal basement membrane of the monkey. But serum E, in contrast to serum B, gave positive IF staining on the guinea-pig tissue. All sera tested in this as well as in the preceding studies yielded immunofluorescent staining of the basement membrane of the monkey oral mucosa.

DISCUSSION

It is becoming increasingly evident that the variations in the spectra of specificities of bullous pemphigoid antibodies are quite unpredictable. It is evident from the results of our studies, as well as those reported previously, that no consistent associations exist in the reactivities of these antibodies with different structures in the different species. For example, it is not possible to predict variation in the spectrum of species specificities on the basis of the reaction patterns on different epithelial structures of monkey tissue. These observations suggest the following:

(1) The only generalization that appears to be tenable at present is that all sera which contain demonstrable bullous pemphigoid antibodies appear to yield reactions with oral and oesophageal tissue of primate origin, while reactivity with other species or other primate tissues is variable. Interestingly, the same appears to be true of pemphigus antibodies (Chorzelski and Beutner, 1969; Beutner *et al.*, 1970; Chorzelski and Beutner, 1971; Cormane and Petzoldt, 1970). The major corrolaries to the observed variations in the spectra of specificities of bullous pemphigoid antibodies may be considered from two points of view, the theoretical and the practical. From the theoretical point of view the observed variations in specificities do not preclude the possibility that selective specificities of patient sera for primate hair follicles or skin will be found in the future. This generalization simply indicates that *monkey oral or oesophageal basement membranes appear to be the antigen of choice.*

(2) Similarly, the question arises as to whether the observation that about 30% of sera from clinically diagnosed cases of bullous pemphigoid (confirmed by direct IF studies on biopsies) are truly negative for circulating antibodies, or whether the failure to employ reactive substrate(s) accounts for the observed negative findings. This possibility is supported by an observation on 1 patient with clinically typical bullous pemphigoid where monkey, guinea-pig and rabbit sections failed to reveal the presence of basement membrane antibodies in the serum, but where typical linear staining of the basement membrane was clearly demonstrable by direct immunofluorescent staining adjacent to the lesion. Sections of a normal skin biopsy in which only a weak direct staining reaction of the basement membrane was demonstrable were employed as substrate for an indirect test with the patient's own serum. Interestingly, the reactions in this indirect test were

uniformly strongly positive in contrast to the weak positive staining in the direct test as seen in the saline controls. This finding suggested that the patient's serum contained an antibody reactive with his own skin but non-reactive with all animal tissues tested.

On the other hand, in dealing with sera with broad specificities, it would not be surprising if some antibodies were found which also react with the basement membrane of kidney and thus overlap in their spectrum of reactivity with the basement membrane antibodies of Goodpasture's syndrome. This seems particularly significant since at least some sera have been found to be reactive with bladder and urethral epithelium (Beutner *et al.*, 1965).

(3) From the theoretical point of view it is obvious that the antigens with which these antibodies react should now be identified. It must be kept in mind that, as in the case of pemphigus antibodies (Chorzelski and Beutner, 1969), the observed differences in immunological specificities of bullous pemphigoid antibodies reflect differences in the chemical structure of the antigenic determinants. Thus, in performing biochemical studies on the antigen, it will be necessary to use a battery of sera containing bullous pemphigoid antibodies with diverse specificities.

(4) From the practical point view of testing for bullous pemphigoid antibodies in patient sera it must be kept in mind that the method should detect autoantibodies and that the procedures should be reproducible. Human autopsy tissues are quite variable in their reactivities. The same is true of human biopsy tissue of oral epithelium, since it is usually pathological. Human skin fequently fails to react because of the restricted specificity of pemphigus antibodies. Thus, for routine testing, the stratified epithelium of *monkey mucosa affords the best approximation to the ideal antigen.* In the light of some of the theoretical considerations given above, the biopsies of the patients' own skin might be used as antigen in problem cases in which monkey mucosa fails to reveal the presence of bullous pemphigoid antibodies.

(5) Another practical consideration is that of testing simultaneously for both pemphigus and bullous pemphigoid antibodies. Fortunately monkey oral or oesophageal mucosa is also the antigen of choice for the demonstration of pemphigus antibodies (Beutner *et al.*, 1970). This recommendation contradicts that of Katz, *et al.*, 1969; when recommendation of human epidermis is not tenable in the light of the now well-documented existence of pemphigus antibodies which fail to react with human epidermis but which yield strong reaction with monkey mucosa. Thus, the antigen of choice for both the pemphigus antibodies and the bullous pemphigoid antibodies appears to be monkey mucosa.

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DEMONSTRATION OF INTERCELLULAR IMMUNOFLUORESCENCE AND EPIDERMAL HYSTERESIS IN BULLOUS FIXED DRUG ERUPTION DUE TO PHENOLPHTHALEIN

WALTER B. SHELLEY, OTTO L. A. SCHLAPPNER AND HAROLD B. HEISS*

Summary.—We have reported a case of severe bullous erythema multiforme type of fixed drug eruption in which (1) multiple drugs were erroneously first indicated as the cause; (2) a false positive bullous patch test was obtained and discovered to be an intracorneal artefact, reflecting a new phenomenon, epidermal hysteresis; (3) a relationship to auto-sensitization and pemphigus was discerned by the demonstration of direct intercellular immunofluorescence in the lower epidermis of the affected fixed sites only; (4) phenolphthalein was finally proven to be the specific cause by double-blind oral challenge.

MODERN immunofluorescent staining techniques have gained for us new insight into the pathogenesis of bullous and auto-immune diseases. The present study describes a unique extension into the field of the fixed drug eruption. It also underscores the need for: a constant doubt as to the completeness of the patient's historical data; a scepticism of the clinical reading of the patch test response; an awareness of how events in the past month may condition the response of the patient's epidermis in a completely non-immunological fashion.

CASE REPORT

A white woman aged 22 was admitted to the Hospital of the University of Pennsylvania for evaluation and treatment of extensive inflammatory and bullous lesions present for 5 days on the lips, mouth, tongue and hands. She gave a history of having had identical lesions in precisely the same sites 3 times previously. In each case the onset, appearance and symptomatology were similar, but progressively more severe. The swelling of the tongue and oropharynx developed to the point of partial airway obstruction.

In June 1970, after taking a sulphonamide preparation for 2 days for an upper respiratory tract infection, she developed swelling of her tongue, and localized red, round, burning patches on her lips, tongue, hands and wrists. After healing, a residual blue-grey pigmentation remained at the previously involved sites.

In November 1970, after taking aspirin for 3 days, she developed a similar, but more severe, reaction. The lesions at this time became bullous. In addition she noticed a burning sensation in the vulvar area.

The patient's third major attack began on February 18th, 1971. Early in February, after 3 days of right-sided pleuritic pain and a mild fever as the only symptoms, a right-sided pneumonia was diagnosed clinically and by chest X-ray. In the subsequent 2 weeks and prior to the explosive appearance of the bullous eruption, she had been given the following sequence of medication: procaine penicillin G, ampicillin, benzathine

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* Postdoctoral trainee under Public Health Service grant TI AM 05261.

penicillin, tetracycline and potassium phenethicillin. Potassium phenethicillin was the suspected offender since it had been given just 5 hours before the onset of this new attack, which was heralded by burning lips, a swollen tongue and reddening of the previous sites of involvement. Within 2 days bullae were present at all sites.

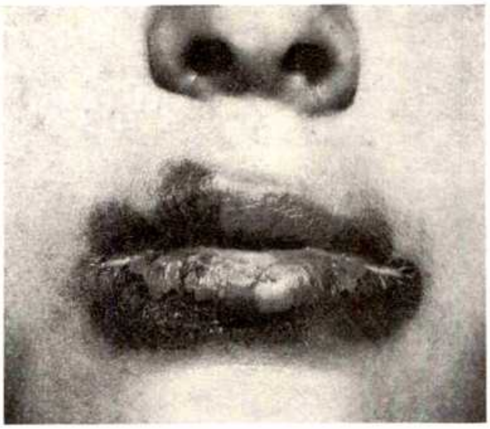

FIG. 1.—Erosive cheilitis and pigmented fixed drug eruption of lips 5 days after taking 300 mg of yellow phenolphthalein in oral laxative (Feenamint®). Entire oral mucosa and tongue were inflamed and showed numerous erosions.

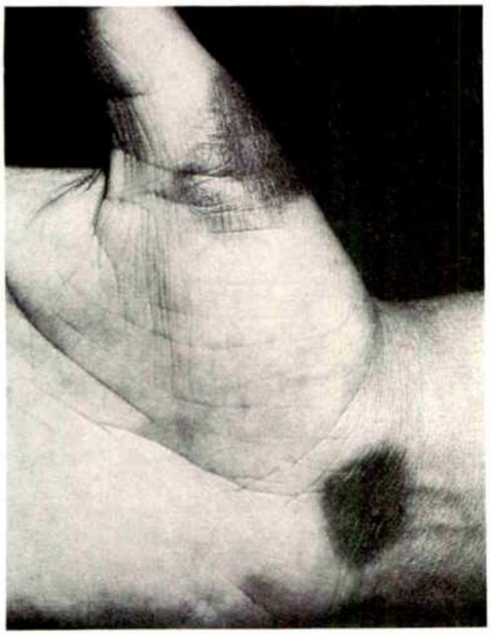

FIG. 2.—Flaccid bullae over sharply circumscribed pigmented plaques of hand as seen at the same time as Fig. 1.

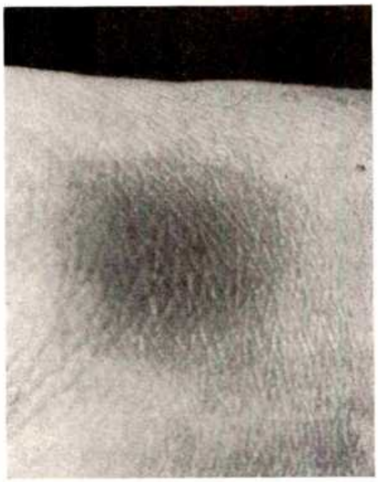

FIG. 3.—Appearance of eruption between attacks. Biopsy of this showed IgG and C3 complement by direct immunofluorescence in the basal and supra-basal portion of the epidermis.

Positive past history consisted of (1) occasional headaches, relieved by salicylate compounds; (2) recurrent mild cystitis, treated mainly with sulphonamides; (3) spastic colon, treated with antispasmodics.

Family history revealed a consanguineous gipsy ancestry. The patient's mother, 1 sister, paternal grandmother and a paternal great aunt all had experienced bullous reactions which had been ascribed to penicillin sensitivity.

Positive findings on physical examination were: several old dry bullae on the outer lower lip (Fig. 1), flaccid grey circular bullae on the inner lips, erosions on the floor of the mouth, a swollen eroded tongue, a reddened pharynx, 2 well-defined, circular, purple oedematous plaques, and 4 flaccid, greyish-blue bullae on the fingers, hands and right wrist. These were 1–2 cm in size (Fig. 2, 3).

Cervical lymph nodes were slightly enlarged bilaterally and tender. Temperature was 37·6°C.

The patient responded to therapy with prednisone in decreasing doses. The bullous skin lesions healed without ulceration but retained a characteristic bluish-grey pigmentation, which was even more marked 3 months later.

Laboratory studies.—Haemoglobin 12·2–14·9 g%, haematocrit 42–46, WBC 12,700 to 18,800/mm^3, neutrophils 63–80%, eosinophils 1–3%, monocytes 2–9%, lymphocytes 18–27%, platelets 400,000.

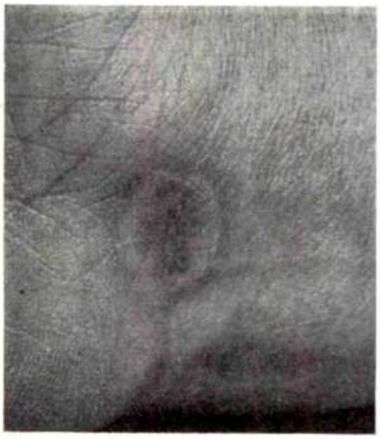

FIG. 4.—Bulla produced by occlusive patch testing to phenethicillin at fixed site on right wrist 16 days after a clinical attack. Histological study proved this to be false positive test (Fig. 5, 6).

Fasting blood sugar, blood urea nitrogen, serum electrolytes and urinalysis were all normal. RPR card test was non-reactive. Total protein—7·7 g/100 ml, with an increase in the α-1 (0·31 g%), α-2 (1·02 g%) and β-globulin (1·06 g%) fractions. Serum immunoelectrophoresis was normal, as were ECG, chest, gall bladder and upper G.I. X-rays. Serum complement studies were normal. No cold agglutinins were demonstrable. Tzanck smears and KOH examinations revealed no evidence of viral or fungal infection.

Closed patch tests were performed by applying directly water-moistened powders of aspirin, sulphathiazole estinyl oestradiol, progesterone, potassium phenethicillin, ampicillin, benzathine penicillin G and penicillin G to normal and the previously involved skin. At 36 hours a flaccid bulla (Fig. 4) had developed at the potassium phenethicillin fixed drug test site, but not at the normal skin test site. This was read as a positive bullous patch test by 3 experienced observers. The other test sites and all control sites showed no reaction.

Histology.—A biopsy of the positive patch test site showed an intracorneal bulla, whose lumen was filled with erythrocytes and occasional lymphocytes and aggregations of necrotic epithelial cells, some of which retained their nuclei (Fig. 5, 6). Acanthosis and spongiosis of the epidermis, as well as small focal areas of liquefaction degeneration of the basal cell layer were present. Oedema, a mild perivascular lymphocytic infiltrate and heavily pigmented macrophages were seen in the upper dermis (Dr Margaret Gray

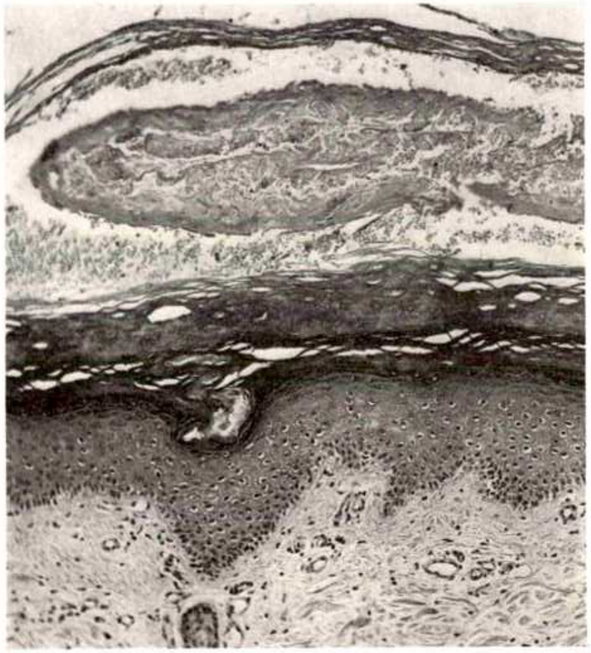

FIG. 5.—Histological pattern of bullous patch test seen in Fig. 4 shows it to be intracorneal. (×135.)

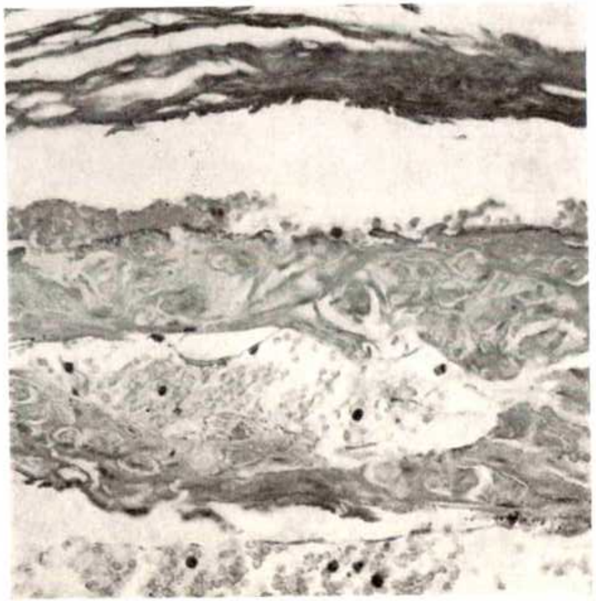

FIG. 6.—Higher power view of contents of the bulla in Fig. 5 reveals aggregates of necrotic epithelial cells. This suggests that the intracorneal bulla of the test resulted from occlusion and hydration of a defective stratum corneum, i.e. precisely at the time when it was composed of the keratinocyte population damaged in the clinical bullous reaction of 16 days prior. Control occlusive patch tests with phenethicillin at a fixed site 21 days post-clinical, as well as on normal skin, were negative. Similar patch tests to phenolphthalein were likewise negative. (×385.)

Wood). Direct basophil tests for potassium phenethicillin, penicillin G and aspirin sensitivity were negative.

Immunofluorescent Studies

Materials and methods.—Shave biopsies were obtained from involved and normal skin, snap frozen in liquid nitrogen, embedded in OCT medium, sectioned at 4 μm in a cryostat at 20°C, and placed on glass slides. These were washed in phosphate buffer at pH 7·2 for 5 min, fixed in acetone for 10 min and air dried.

Specimens for the direct immunofluorescent studies were overlaid with goat-derived, fluorescein-tagged, anti-human IgG, IgA, IgM conjugates and C3 complement* and diluted 1 : 4 with phosphate-buffered saline containing 2% Tween 80®.

After incubation for 30 min at 37°C, the specimens were given 10-min washes in phosphate-buffered saline and a final rinse with distilled water.

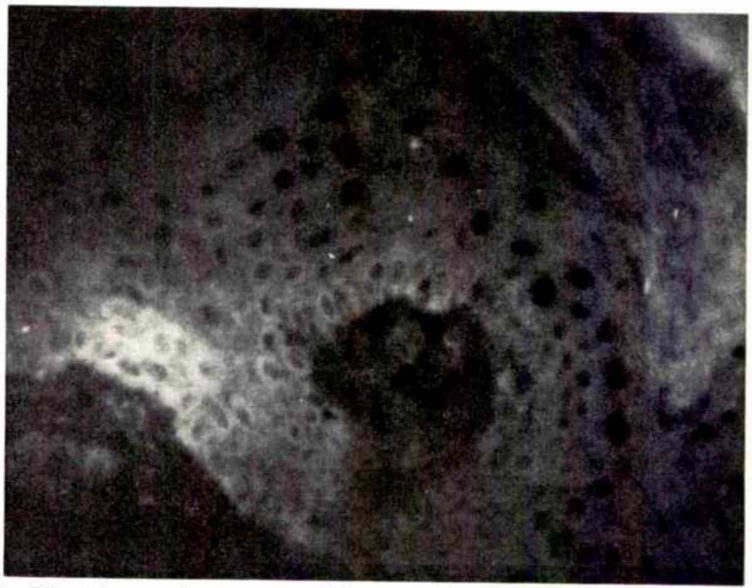

FIG. 7.—Direct immunofluorescent staining for IgG. Intracellular immunofluorescence of lower epidermis of lesion seen in Fig. 3.

The slides were then cover-slipped, using FA mounting media, and examined with a Leitz Ortholux microscope using a BG 12 Schott exiter filter and a K530 Leitz barrier filter.

Sections for indirect studies consisted of horizontally cut fresh normal human epidermis sectioned at 4 μm. These were overlaid with the patient's serum diluted 1 : 10 with phosphate-buffered saline containing 2% Tween 80, incubated for 30 min at 37°C, and subsequently given three 10-min washes in phosphate-buffered saline. They were then overlaid with goat-derived, fluorescein-tagged anti-IgG, anti-IgA, anti-IgM conjugates and C3 complement. The second part of the procedure was the same as that described for the direct method.

Normal controls for the direct studies consisted of the patient's normal skin processed as described for the direct studies. Controls for the indirect studies consisted of normal serum and known positive sera of patients with pemphigus foliaceus, bullous pemphigoid, and systemic lupus erythematosus on horizontally cut 4 μm sections of fresh normal human epidermis.

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Results.—Intercellular immunofluorescence of moderate intensity was seen in the basal cell layer and the 2–3 adjacent suprabasilar cell layers of the involved epidermis with the anti-IgG conjugate (Fig. 7) and C3 complement.

Anti-IgM and anti-IgA gave negative results, as did the patient's direct control studies and all the indirect studies. In none of the sections was dermo-epidermal or anti-nuclear fluorescence seen.

Post-hospitalization Course

Discharged after 2 weeks of hospital study and treatment, the patient had only residual hyperpigmented greyish-brown macules on her lips, hands and arms. She was cautioned to avoid penicillin of all types as well as sulphonamides and compounds containing salicylate.

In May 1971, a fourth attack occurred with rekindling of fixed sites as well as a stomatitis, vaginitis and proctitis. Not seen by us, she was given steroids, and the episodes were related to a bowel antispasmodic, dicyclomine hydrochloride (Bentyl®), which she had taken for the week before the attack for the relief of crampy abdominal pain.

At 5 a.m. on June 27th, 1971, the patient was awakened by the onset of yet another attack, which became so severe as to require prednisone in a dose of 160 mg/day. With oral mucosae completely denuded, unable to eat, and with difficulty in breathing, she presented the picture of the Stevens–Johnson syndrome. She attributed the recurrence to tincture of belladonna, which she had been taking for the previous 3 weeks. Finally, by the dint of questioning related precisely to the 6 hours just prior to this attack, it was possible to learn that she had taken 3 laxative tablets (Feenamint®), on retiring on the night of June 26th. She then also recalled that, antecedent to one of the previous attacks, she had taken another proprietary laxative, which also proved to contain phenolphthalein. The manufacturers reported that each tablet contained 100 mg of yellow phenolphthalein.

Occlusive 48-hour patch testing with phenolphthalein powder was negative on the fixed eruption site as well as on normal skin. One month later, blind oral challenge with 25 mg of phenolsulphophthalein (phenol red) produced no reaction, whereas oral challenge with 25 mg of phenolphthalein completely reproduced the bullous fixed drug reaction, with vaginitis, proctitis, stomatitis and bullous lesions at sites of the previous attacks. The onset of symptoms was 10 hours after challenge.

By avoiding all laxatives, tooth pastes, food colourings known to contain phenolphthalein, the patient remains well to date.

DISCUSSION

The diversity of patterning of the fixed eruption due to phenolphthalein has been extensively reviewed by Welsh (1961). Our patient had not only the classic, persistent, red, brown, blue-grey, and at times purple, macules, but also the bullae of an erythema multiforme. The vesicular bullous lesions rapidly denuded the oral mucosa, leaving erosions and superficial ulcers. Historically, each attack was preceded by a different drug so that the patient and physician became more alarmed by what appeared to be a remarkable example of polysensitivity. None the less the search for the unifying theme went on, only to end with a breakthrough in history taking and the unequivocal reproduction of the syndrome by oral challenge with a small dose of phenolphthalein. The specificity of the patient's response was documented by an absence of reaction to phenolsulphophthalein which differs from phenolphthalein only by the presence of an SO_2 instead of a CO grouping in the phthalic anhydride portion of the molecule. The oral challenge was employed since it is the only adequate method of demonstrating fixed phenolphthalein reactions. Fixed site patch testing is of value for certain other drug allergens, but not for phenolphthalein (Welsh, 1961).

In this regard the finding of a positive bullous patch test to sodium phenethicillin deserves attention. Although clinically acceptable, the bulla proved on histological examination to be within the stratum corneum and hence not a replica of the primary clinical event. Repeat testing of old sites and control skin failed to reproduce the phenomenon. We assume that the bulla induced was the result of occlusion and wetting of an area at the exact time when the previously damaged keratinocytes of the clinical bulla had now moved out to comprise the stratum corneum. It was this defective stratum corneum which was splitting in response to hydration and sweat retention (Fig. 5, 6). Thus the patch test bulla was an artefact which reflected the prior events in the epidermis. Such a transitory epidermal conditioning is analogous to the phenomena in physics which reflect the previous history of the materials concerned; as such it could be appropriately labelled: epidermal hysteresis.

Our positive direct immunofluorescent studies indicate intercellular fixation of IgG and C3 complement in the basal and supra-basal portions of the strangely limited islands of epidermis. This is compatible with the hypothesis that phenolphthalein acting as a hapten has chemically reacted with an intercellular epidermal protein of specific areas to form an immunogen. Relevant to this is the observation that, in a tetracycline eruption, lesions showed fluorescence under a Wood's light, suggesting the presence of the drug in the involved tissue (Winter and Freund, 1969). In our patient's fixed drug eruption, induction and binding of specific antibodies, fixation of complement and the ensuing allergic tissue damage could explain the localization of the well-known microscopic (Komura *et al.*, 1969) as well as our new immunofluorescent findings. The characteristic clinical pigmentary change would thus reflect an immune process taking place precisely in the zone of melanocyte activity, but only in fixed sites.

The way our direct immunofluorescent findings relate to those seen in pemphigus vulgaris must remain speculative (Beutner, 1971). In this regard, Beutner *et al.* (1970) have expressed the view that Butazolidine and other drugs " may complex with the intercellular antigen of stratified epithelium to form an altered sensitizing antigen " and thereby induce the autosensitization of pemphigus. They cite 3 instances in the literature of such possible drug provocation of pemphigus antibody formation. Recently Fellner and Prutkin (1970) and Go (1969), using indirect immunofluorescent methods, found anti-epithelial antibodies in the serum of patients with eruptions due to penicillin and sulphamethoxypyridine, respectively.

Our patient, however, had no circulating auto-antibodies and hence was not showing an auto-immune phenomenon. The results we obtained most closely resemble those reported by Brehm and Korting (1970). Using *direct* staining techniques, they observed intercellular immunofluorescence in the granular layer of the involved epidermis in a bullous non-fixed eruption from nalidixic acid. In our patient, the fluorescence was not in the granular but in the basal area, again in keeping with the histological features of a fixed eruption (Montgomery, 1967).

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THE GLANDS OF MONTGOMERY

WILLIAM MONTAGNA AND JEUNG SOON YUN

Summary.—The ducts of the glands of Montgomery, usually in conjunction with sebaceous follicles, produce tubercles at the surface of the areola. The ducts and their glands are similar in every respect to those that open at the tip of the nipple, except that the former are not surrounded by nerve end-organs. We therefore suggest that Montgomery glands are integral parts of the mammary system. In all of the numerous areolae observed, we found only a single, minute hair follicle, and no apocrine sweat glands. Those " glands " previously described as apocrine, whether in the nipple or the areola, are actually the tortuous lactiferous sinuses.

IN this communication we clarify the confusion that persists about the cutaneous appendages in the areola of the breasts, especially the glands of Montgomery, which have been variously referred to as minute (Cummins, 1968) or accessory mammary glands (Giacometti and Montagna, 1962), or intermediates between mammary and sweat glands (Bloom and Fawcett, 1968). We find that these glands are true mammary glands whose ducts and secretory parenchyma are identical with the other mammary glands. Since they are often functional during lactation, they must be considered integral elements of the lactiferous apparatus.

MATERIALS AND METHODS

This study is based on a number of tissues taken from 12 women aged 14, 23, 31, 34 and 49, a few hours after accidental death. In each case, the tissue from 1 breast was treated with sodium bromide (Staricco and Pinkus, 1957) to split the epidermis, which was then dehydrated, dried, and mounted on slides underside up, without a cover slip. The tissue from the other breast was used for the histological preparations. Parts were fixed in 10% formalin, embedded in paraffin, and stained with HE, toluidine blue, periodic acid-Schiff and acid-orcein Verhoeff. Unfixed tissues were cut in the cryostat, and both thick and thin sections were fixed in 10% formalin for 2 hours before being treated with the modified cholinesterase technique of Roman *et al.* (1969) and for alkaline phosphatase (Gomori, 1952). The ducts were traced to glands in serial sections made from one entire areola of a woman aged 23.

OBSERVATIONS

The areolar tubercles that surround the nipple are the sites of the ducts of Montgomery and sebaceous glands (Fig. 1). The tubercles vary in size and conspicuity with each individual and function of the mammary system. The ducts of the glands of Montgomery are normally found, together with sebaceous glands,

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throughout the areola. In split skin preparations the underside of the areolar epidermis has the characteristic patterns described by Montagna (1970). In such preparations the ducts of the Montgomery glands are clearly seen, and sebaceous and some sweat glands are often preserved intact (Fig. 2). Although the secretory parts of the Montgomery glands, which are deeply embedded in the tissue, are mostly broken off, the ducts can be unerringly recognized, arranged in loose groups of 3 to 6, usually accompanied by large sebaceous glands; an eccrine sweat gland is occasionally present somewhere in or near the groups. The groups are arranged in various patterns: some form a circle around a large sebaceous gland (Fig. 3); others are aligned in a row, with a sebaceous gland at 1 or each end, and a few grow singly (Fig. 4). The ducts of the sebaceous glands often empty near or into the ducts of the Montgomery glands (Fig. 5). Some groups have no sebaceous glands near them, and some large sebaceous follicles grow independently of the Montgomery ducts. Six to 10 groups of Montgomery ducts are sometimes randomly distributed within the two-thirds of the areola that immediately surround the nipple (Fig. 2). The only appendages between these groups are single eccrine sweat glands. The periphery of the areola is marked by a ring of hair follicles of different sizes, their sebaceous glands, and some eccrine sweat glands.

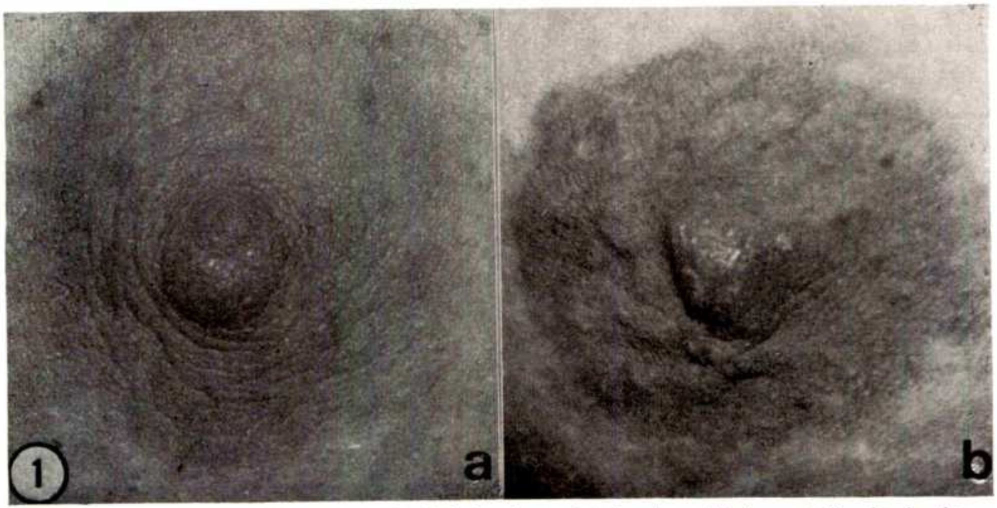

FIG. 1.—(a) Nipple and areola, with slightly elevated tubercles. (b) Large tubercles in the areola of a lactating breast.

The ducts of the Montgomery glands vary in width even in the same specimen (Fig. 3, 4). They all have a wide, funnel-like (Fig. 5) mouth 0·5 mm or less in diameter and penetrate 4 mm or more below the surface before they branch. Unlike the lactiferous ducts in the nipple, the ducts of the Montgomery glands branch deep in the dermis. The branches sometimes coil before becoming dilated into lactiferous sinuses, which in sections resemble apocrine glands (Fig. 5, 7). The thick, funnel-like upper part of the ducts is lined by keratinizing stratified epithelium, and keratin plugs often fill the lumen. Deeper down in the dermis the coiled ducts are lined by either cuboidal or columnar cells, with myo-

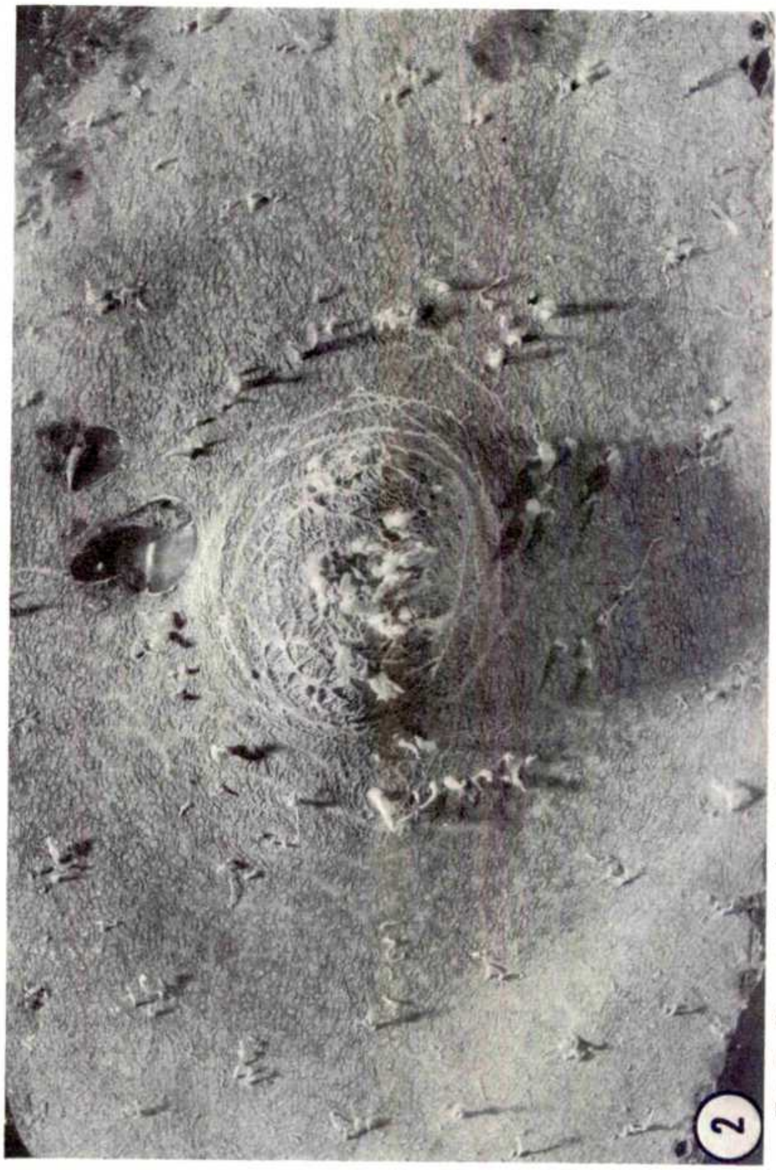

FIG. 2.—The underside of a nipple and areola of a woman aged 23 in a split skin preparation showing the distribution of the ducts of the glands of Montgomery. (×4.)

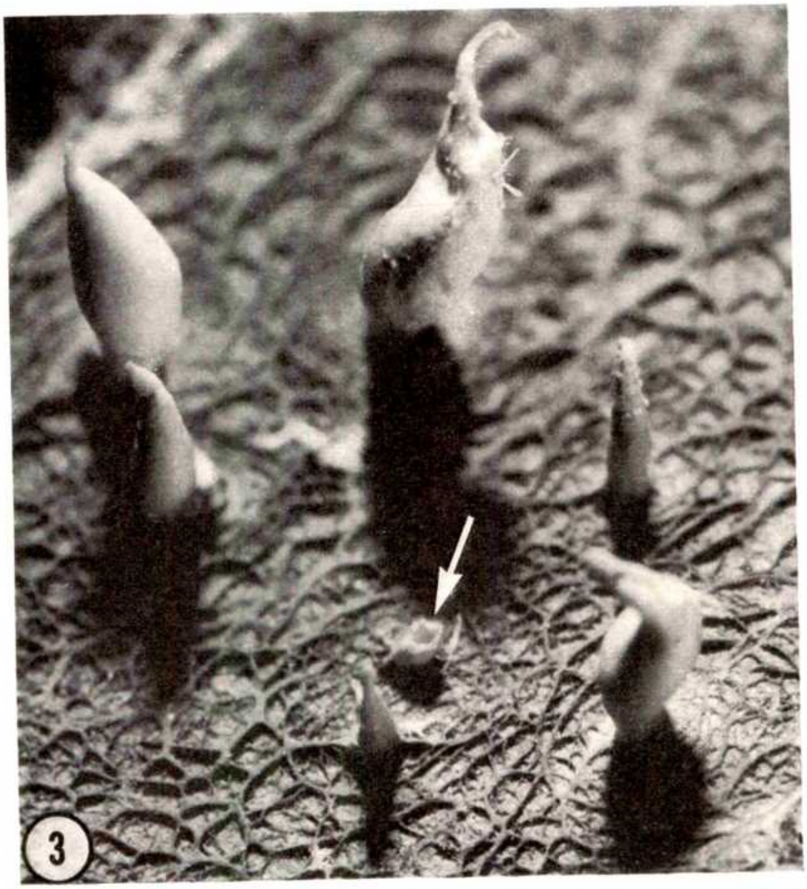

FIG. 3.—Clusters of Montgomery ducts around the duct (arrow) of a sebaceous gland. Split skin preparation. (c. × 125.)

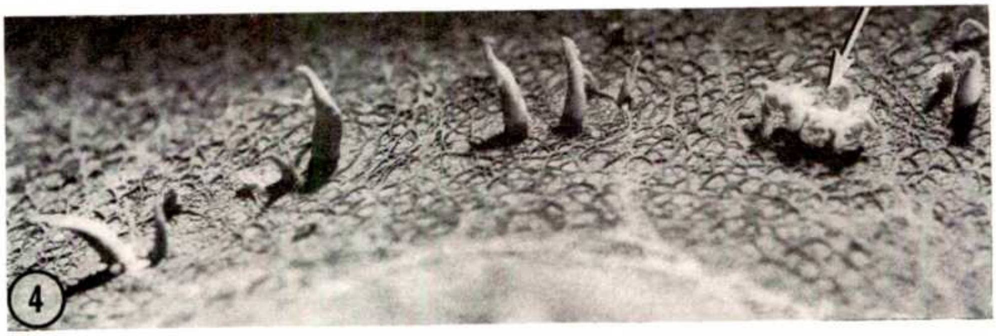

FIG. 4.—Montgomery glands of different sizes arranged in a row, with a fragment of a sebaceous gland (arrow). Split skin preparation. (c. × 112.)

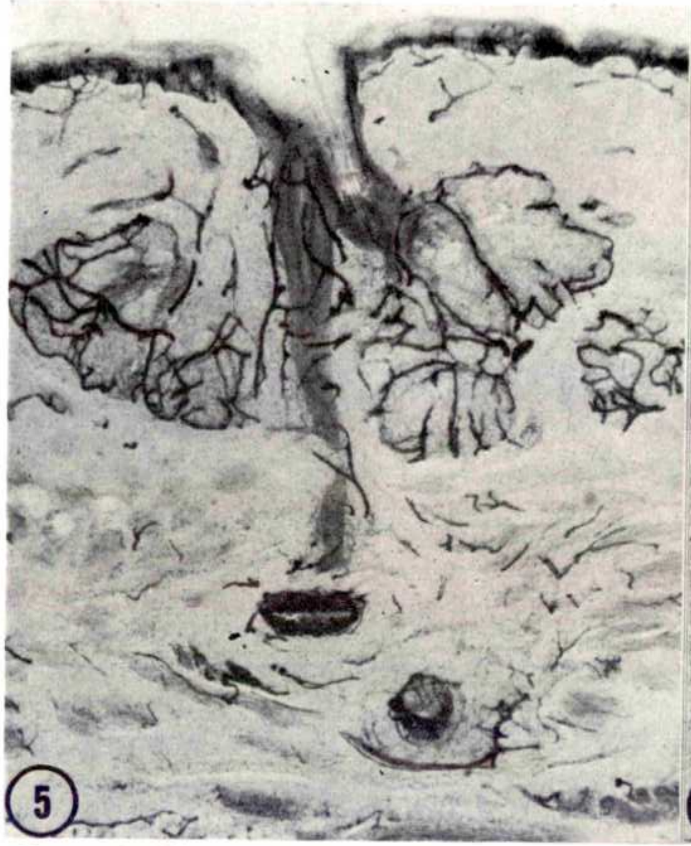

FIG. 5.—A richly vascularized sebaceous gland opening into a duct of Montgomery. The two sections of the lactiferous sinus at the bottom of the figure are continuous with the duct. A miniscular vellus hair, the only one we have found in the areolae we have examined, is barely visible inside the sebaceous duct. Frozen section treated with alkaline phosphatase. (*c.* × 150.)

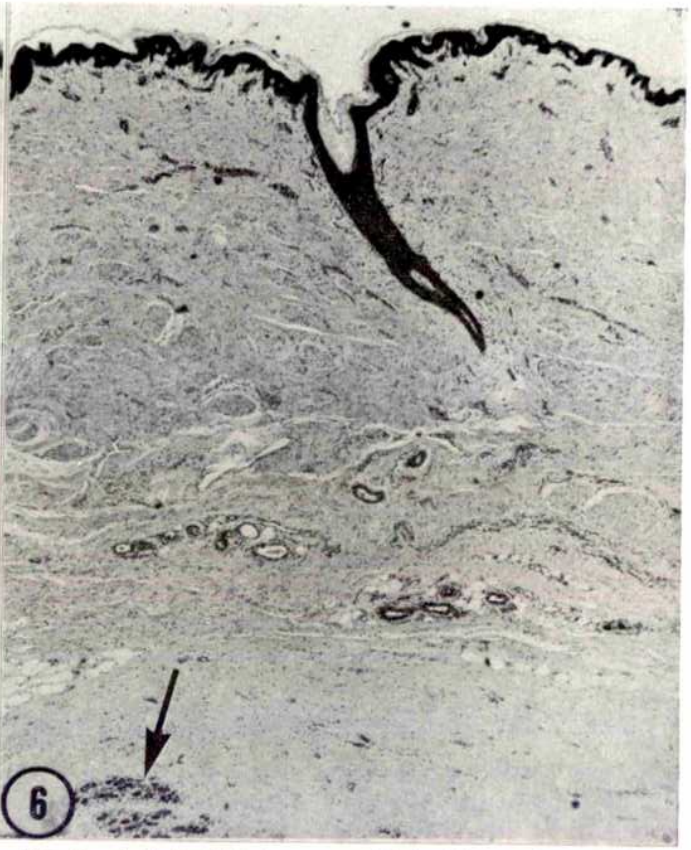

FIG. 6.—A duct of Montgomery with lactiferous sinuses below. In serial sections the duct was traced to the sinuses and the mammary tissue below (arrow). Frozen section stained with toluidine blue. (*c.* × 100.)

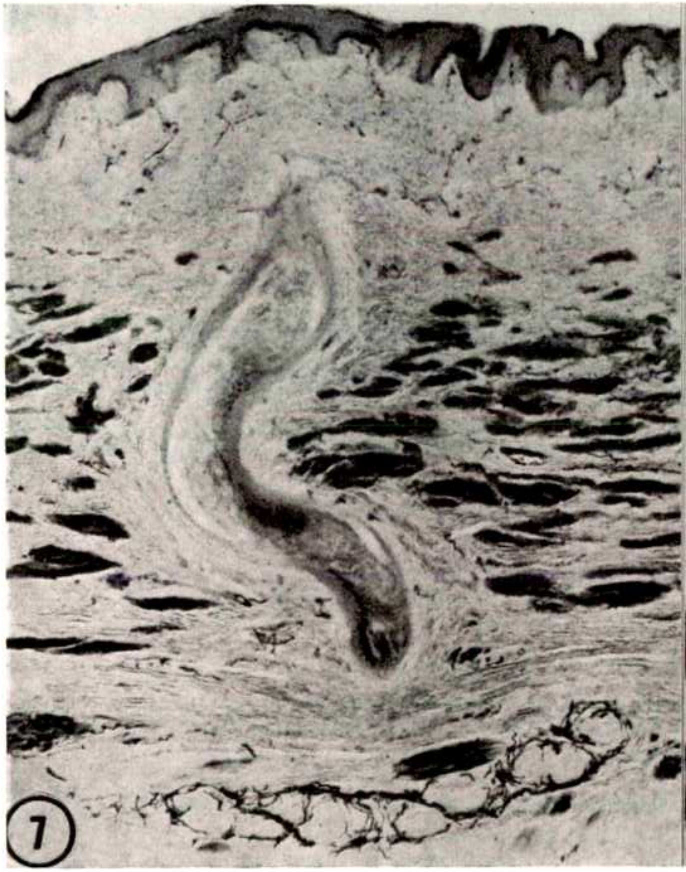

FIG. 7.—A duct of Montgomery and its coiled lactiferous sinus below. The sinuses are surrounded by acetylcholinesterase-reactive nerves. Technique of Roman *et al.* (1969). (*c.* × 200.)

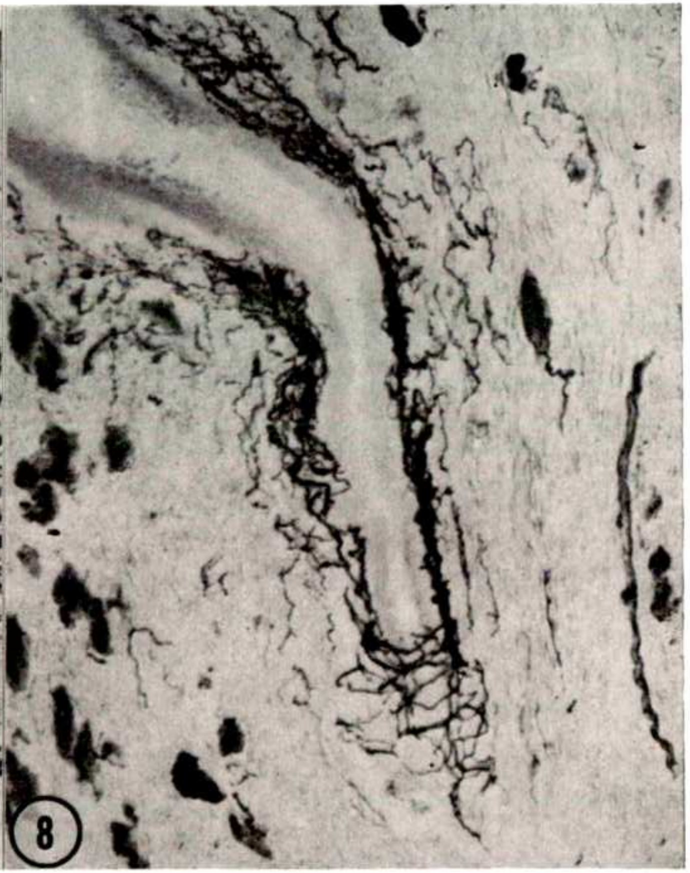

FIG. 8.—Numerous acetylcholinesterase-reactive nerves around a duct of Montgomery. This is similar to the lactiferous ducts that open at the tip of the nipple. Technique of Roman *et al.* (1969). (*c.* × 255.)

epithelial cells at their bases. Around the thick basement membrane of the sinuses and alveolar ducts are variable numbers of delicate elastic fibres similar to those found around sweat glands (Montagna and Giacometti, 1969). In the upper part of the ducts, as in the lactiferous ducts of the nipple (Montagna, 1970), the epithelium includes numerous large dendritic melanotic melanocytes (Fig. 8).

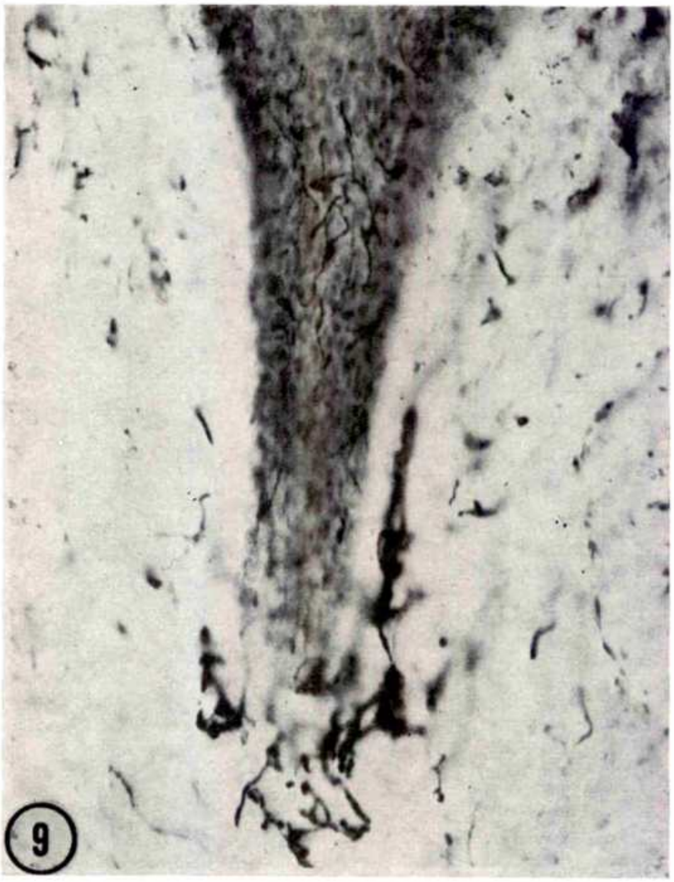

FIG. 9.—Melanocytes around a duct of Montgomery. Technique of Roman *et al.* (1969) for acetylcholinesterase. (*c.* × 255).

The entire duct system and some of the alveoli are conspicuously enwrapped by cholinesterase-reactive nerves (Fig. 8) similar to those described in the nipple. Unlike the nipple, the terminal part of the duct contains no recognizable nerve end-organs. The ducts, particularly the sinuses, are surrounded by numerous capillaries. The glandular alveoli, like those of the rest of the breast tissue, are reactive for alkaline phosphatase (Giacometti and Montagna, 1962).

Montagna (1970), who described the sebaceous glands, called attention to the predictable presence of melanocytes in them.

With one exception (Fig. 5) no hair follicles were found in the areola, but follicles are always present at the periphery of the areola.

DISCUSSION

The major point of this report is that the glands of Montgomery are integral parts of the mammary apparatus. The structure of the ducts and of the parenchyma of the glands is similar in every respect to that of the glands that emerge at the nipple. In both, the ducts and sinuses are richly vascularized and innervated and, in both, the ducts are copiously supplied with dendritic melanocytes. The only difference is the absence of tactile end-organs around the orifice of the ducts of the Montgomery glands. These glands are neither small nor accessory, but are a part of the total breast tissue. Furthermore, they often become functional during lactation and milk can be expressed from them.

Although we have searched for apocrine glands in more than a dozen specimens, we have found none. The " glands " previously identified by other authors as apocrine are really the tortuous lactiferous sinuses of the ducts. We suggest that apocrine glands in nipples and areolae are at best a rare finding.

We have also ascertained here that, in the areola, hair follicles are absent or rare. We found only 1 in the middle of a sebaceous follicle, so small that the vellus hair it produced was about 5 μm in diameter. The periphery of the areola, however, is always ringed by hair follicles that are large or small, depending on the individual.

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THE ACTIVITY OF METABOLIC ENZYMES OF HUMAN EPIDERMIS IN RELATION TO AGE

S. YAMASAWA*, D. CERIMELE AND F. SERRI

Summary.—The activity of 14 enzymes, representative of the main metabolic pathways, was studied in the epidermis of 63 normal human subjects ranging in age from 1 month to 90 years. The activity was assayed with microfluorometric methods which allow the use of samples of epidermis very much reduced in size (0·3 μg to 1 μg). No difference of activity was observed in any of the enzymes studied despite the varied ages. The lack of influence of age on the activity of the enzymes in human epidermis enhances the significance of the variations which are reported in some pathological conditions (like psoriasis, chronic sun-damaged skin and neoplasms).

SOME interesting alterations observed in the activity of metabolic enzymes of the epidermis from sun-exposed areas of the body of aged subjects (Cerimele *et al.*, 1971) induced us to study the impact of ageing on the activity of these enzymes.

The main clinical, histological and biochemical alterations observed in the skin of old humans have been recently reviewed (Serri, 1969; Cerimele and Serri, 1969; Cerimele and Serri, 1971). Only scanty studies have been done previously on the influence of age on the level of activity of some metabolic enzymes in the human skin. According to some reports, the activity of most enzymes is decreased by 15 to 25% after the 60th year (Salfeld, 1966); other studies did not find any significant alterations (Serri and Rabbiosi, 1956; Ribuffo, 1967).

These contrasting results prompted us to undertake the present study on the influence of age on the activity of a significant group of metabolic enzymes of the human epidermis.

MATERIALS AND METHODS

Skin biopsies were obtained from 63 healthy human volunteers ranging in age from 1 month to 90 years (32 were males and 31 were females). The distribution of the subjects in different age groups was carefully planned according to a statistical design so that each decade was represented by at least 6 people. For the statistical evaluation the subjects were separated into 4 groups according to age: 0–20, 20–40, 40–70, 70–90.

From each subject a biopsy was obtained from the lower left part of the abdomen, either using an 8 mm electric punch or during surgical interventions.

The biopsies were immediately frozen in solid carbon dioxide, and then cut 20 μ in thickness in a cryostat at −20°C. The frozen sections were dried *in vacuo* to preserve the activity of the enzymes. The epidermis was dissected with a free-hand knife under a dissecting binocular microscope, and small samples (0·3–1 μg) were weighed with a

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* Gillette Fellow.

quartz fishpole balance and then transferred into small test tubes (Lowry, 1953). The activity of the enzymes studied was assayed with fluorometric methods (Lowry *et al.*, 1957).

Fourteen enzymes, representative of the glycolytic pathway, tricarboxylic acid cycle, the transamination linkages between amino-acid and carbohydrate metabolism, the

TABLE I.—*Enzymes Studied*

(1) Glycolysis	
(1) Hexokinase	(Adachi and Yamasawa, 1966a)
(2) Phosphofructokinase	(Adachi and Yamasawa, 1968)
(3) α-Glycerophosphate-dehydrogenase	(Adachi and Yamasawa, 1966b)
(4) Glyceraldehyde-3-phosphate dehydrogenase (G 3-PDH)	(Im *et al.*, 1966)
(5) Enolase	(Adachi and Yamasawa, 1966c)
(6) Lactic dehydrogenase	(Im and Adachi, 1966b)
(II) Tricarboxylic acid cycle	
(7) Isocitric dehydrogenase (ICDH)	(Adachi, 1968)
(8) Malic dehydrogenase (MDH)	(Adachi, 1968)
(9) Malic enzyme (ME)	(Adachi, 1968)
(III) Amino acid metabolism	
(10) Alanine transaminase	(Adachi *et al.*, 1967b)
(11) Aspartate transaminase	(Adachi *et al.*, 1967b)
(IV) Pentose phosphate shunt	
(12) Glucose-6-phosphate-dehydrogenase (G-6-PDH)	(Im and Adachi, 1967a)
(13) 6-Phosphogluconic-dehydrogenase (6-PGDH)	(Im and Adachi, 1967a)
(V) Fatty acid metabolism	
(14) β-Hydroxylacyl-dehydrogenase (B-OH-DH)	(Adachi *et al.*, 1967a)

TABLE II.—*Means of the Activity of* 14 *Enzymes of the Epidermis of* 63 *Subjects Ranging from* 1 *Month to* 90 *Years of Age. In Each Group of Age There Are At Least* 6 *Males and* 6 *Females. The Activity is Expressed as mol/kg Dry wt Tissue/h.*

Enzymes		0–20	21–40	41–70	71–90
Hexokinase	M	0·6340	0·5066	0·5830	0·7305
	F	0·7164	0·5602	0·7075	0·6395
Phosphofructokinase	M	0·7210	0·6964	0·6844	1·3382
	F	0·7600	0·7914	1·0228	0·9070
α-Glycerophosphate-dehydrogenase	M	0·1157	0·0896	0·1028	0·1293
	F	0·1227	0·1438	0·1345	0·1091
Glyceraldehyde-3-phosphate dehydrogenase	M	4·0762	3·8720	3·7260	5·9000
	F	5·2100	5·1960	4·8467	4·4425
Enolase	M	7·2662	7·1500	6·4250	8·4033
	F	7·5343	7·8020	7·7617	7·2250
Lactic dehydrogenase	M	18·2350	16·6800	19·6640	25·5329
	F	18·3357	24·4580	22·3517	20·7800
Isocitric dehydrogenase	M	1·2065	1·2272	1·5221	1·4029
	F	1·2897	1·7096	1·5183	1·1779
Malic dehydrogenase	M	16·9750	13·6040	16·1289	19·0533
	F	19·2714	24·3060	21·9380	16·3967
Malic enzyme	M	0·2232	0·2148	0·2036	0·1829
	F	0·1956	0·2206	0·1875	0·1592
Alanine transaminase	M	0·8209	0·7238	0·8092	1·0730
	F	0·7893	0·7772	0·8988	0·8772
Aspartate transaminase	M	2·0461	1·7772	1·7321	2·4540
	F	2·1570	1·6766	1·9097	1·8584
Glucose-6-phosphate dehydrogenase	M	0·5354	0·4566	0·5336	0·6277
	F	0·5913	0·6960	0·6395	0·5269
6-Phosphogluconic dehydrogenase	M	0·2391	0·2474	0·2720	0·2900
	F	0·3350	0·2380	0·2108	0·2174
β-Hydroxylacyl dehydrogenase	M	1·9286	1·5428	1·3542	2·1196
	F	1·5329	1·9604	1·7525	1·7236

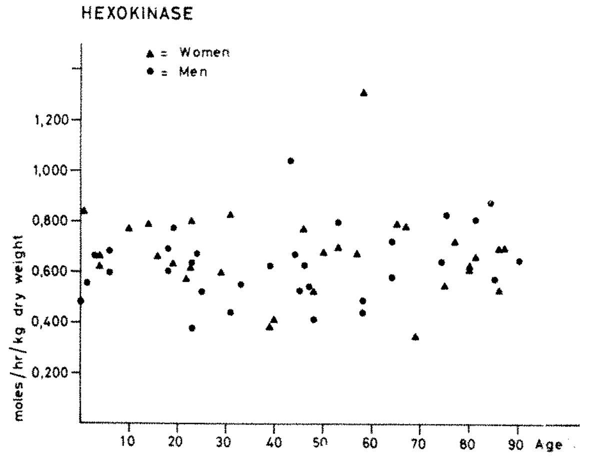

FIG. 1.—The activity of hexokinase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

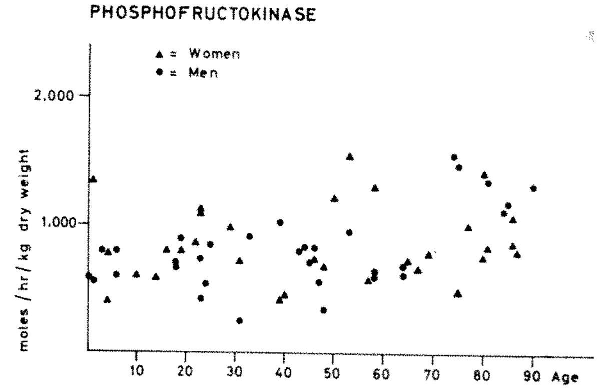

FIG. 2.—The activity of phosphofructokinase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

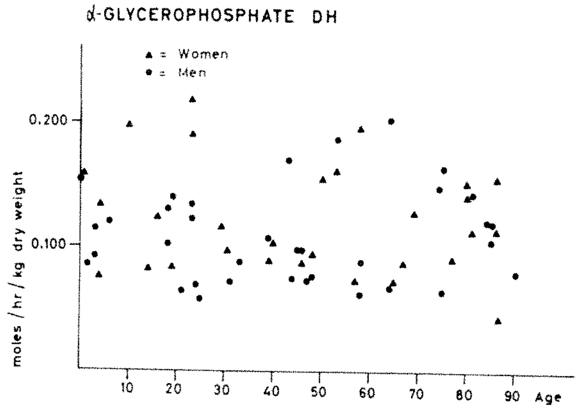

FIG. 3.—The activity of α-glycerophosphate-dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

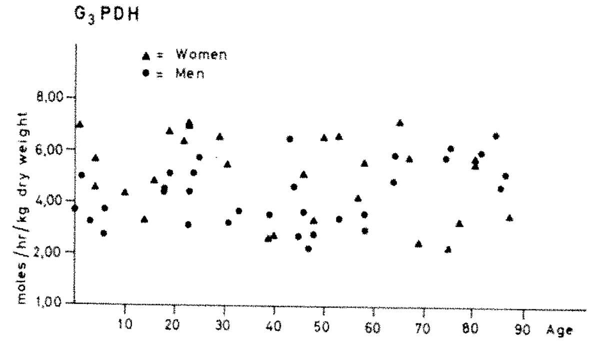

FIG. 4.—The activity of glyceraldehyde-3-phosphate dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

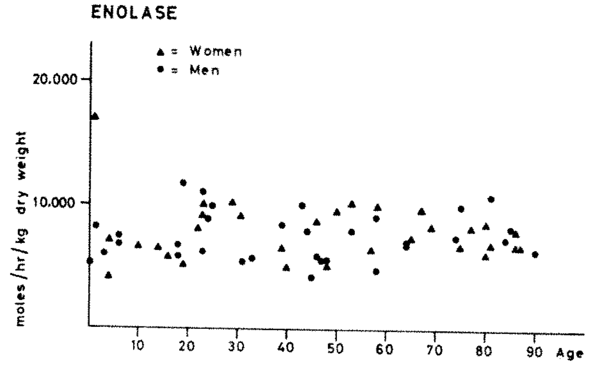

FIG. 5.—The activity of enolase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

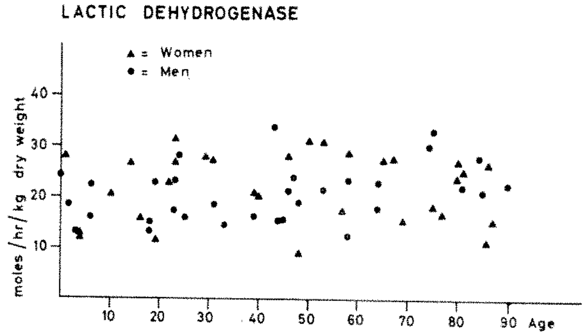

FIG. 6.—The activity of lactic dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

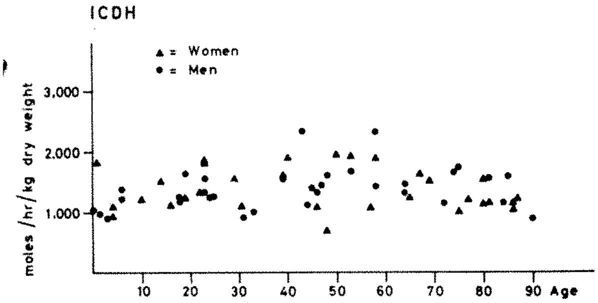

Fig. 7.—The activity of isocitric dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

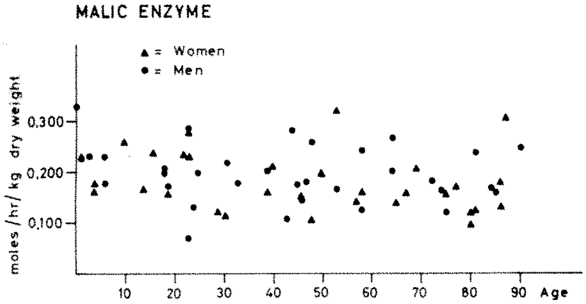

Fig. 9.—The activity of malic enzyme in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

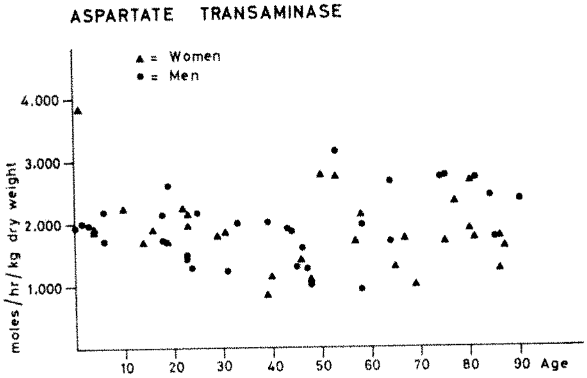

Fig. 11.—The activity of aspartate transaminase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

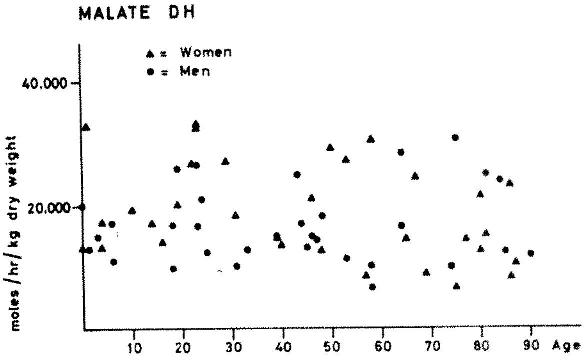

Fig. 8.—The activity of malic dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

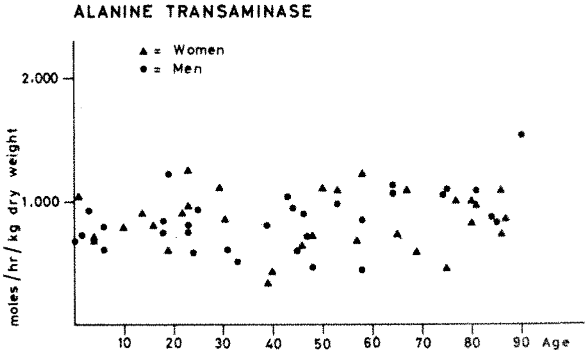

Fig. 10.—The activity of alanine transaminase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

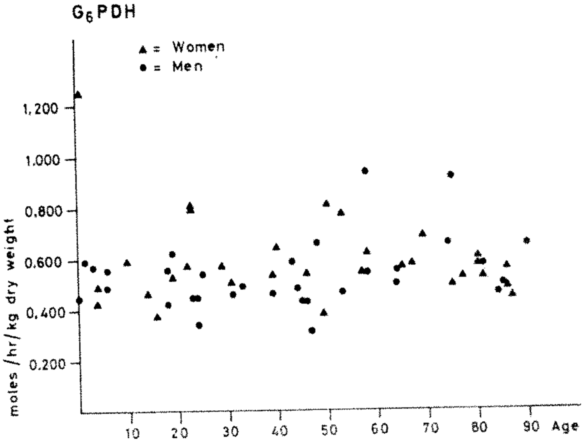

Fig. 12.—The activity of glucose-6-phosphate-hydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

pentose phosphate pathway and fatty acid metabolism were studied. The enzyme activities were assayed with microfluorometric methods as adapted to the skin by Hershey *et al.* (1960). For each assay 5 different samples were run. The detailed methods for the single enzymes have been worked out and described by Adachi *et al.* from the Oregon Primate Centre in the quoted papers (Table I).

RESULTS

The results of the assays of the 14 enzymes we have studied are shown in Figs. 1 to 14 and summarized in Tables II, III. The analysis of variance has demonstrated that the activity of the enzymes studied is not affected by age; moreover no difference between men and women could be demonstrated.

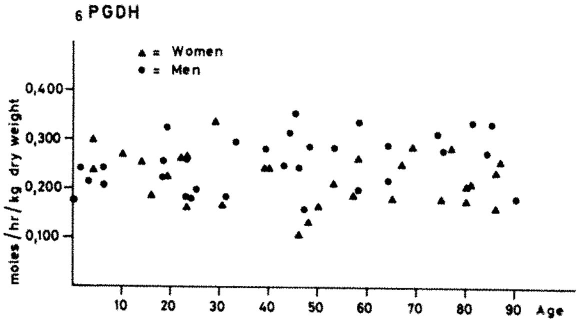
6 PGDH

FIG. 13.—The activity of 6-phosphogluconic-dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

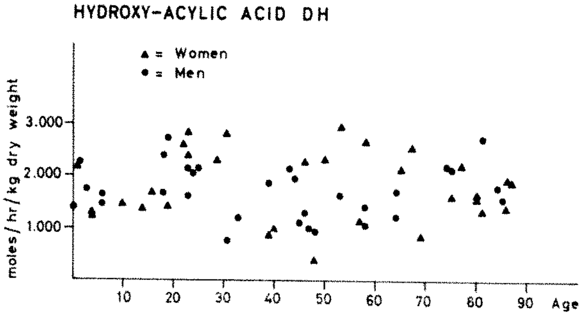
HYDROXY-ACYLIC ACID DH

FIG. 14.—The activity of β-hydroxylacyl-dehydrogenase in the epidermis of 63 healthy human subjects, ranging from 1 month to 90 years of age. The activity is expressed as mol/kg dry wt/h.

TABLE III.—*Means of the Activity of* 14 *Enzymes of the Epidermis of* 32 *Males and of* 31 *Females Ranging From* 1 *Month to* 90 *Years of Age. The Activity is Expressed as mol/kg Dry wt Tissue/h.*

Enzymes	Males	Females	Total
Hexokinase	0·629	0·676	0·652
Phosphofructokinase	0·820	0·856	0·837
α-Glycerophosphate-dehydrogenase	0·111	0·106	0·108
Glyceraldehyde-3-phosphate dehydrogenase	4·213	5·081	4·640
Enolase	7·487	7·858	7·652
Lactic dehydrogenase	20·938	22·253	21·585
Isocitric dehydrogenase	1·423	1·380	1·409
Malic dehydrogenase	16·764	19·494	18·107
Malic enzyme	0·196	0·176	0·187
Glucose-6-phosphate dehydrogenase	0·535	0·591	0·563
6-Phosphogluconic dehydrogenase	0·257	0·245	0·251
Alanine transaminase	0·847	0·815	0·830
Aspartate transaminase	1·936	1·867	1·902
β-Hydroxylacyl-dehydrogenase	1·730	1·834	1·782

DISCUSSION

The activity of succinic dehydrogenase in whole skin was shown to have decreased slightly, but not significantly, in the skin of aged people (Serri and Rabbiosi, 1956).

The study of 17 enzymes of the energy and amino-acid metabolism showed that the activity of most of them in the skin of aged humans (beyond 60 years) decreased by 15 to 25%; hexokinase decreased by about 60%, while glucose-6-phosphate dehydrogenase activity increased by 18%. The activity of the enzymes of the citric acid cycle was lower than the activity of the enzymes of glycolysis; therefore the initial substrate is more likely to be glycogen than glucose, due to the decreased activity of hexokinase. The increased activity of glucose-6-phosphate dehydrogenase was explained by the increased need of pentoses for the synthesis of nucleic acid (Salfeld, 1966).

The study of 3 enzymes: glucose-6-phosphate dehydrogenase, lactic dehydrogenase and aldolase, showed that the activity of glucose-6-phosphate dehydrogenase and lactic dehydrogenase is high in the skin of young humans (until 20 years) then decreases and remains constant throughout the rest of the life, whereas the activity of aldolase is always constant (Ribuffo, 1967).

Consideration of the overall results of our study on the activity of 14 enzymes during life induces us to think that the activity of the metabolic enzymes of human epidermis is not affected by age.

To explain the ageing of the cells 2 main hypotheses have been put forward: the first is called the "error mechanism" theory, and maintains that ageing depends on the cumulation of injuries; the second is called the "ageing programme" theory and affirms that ageing is the consequence of some programmed morphological or functional alterations (von Han, 1970). Our results, showing that no specific enzymatic alteration related to ageing could be demonstrated, are therefore more in agreement with the first hypothesis than the second.

The results of the present study confirms the opinion expressed by some authors (Ribuffo, 1967) that the efficiency of glycolysis and of the pentose phosphate pathway is still necessary for the skin of old people. Our results are also important for researches on enzyme activity in some pathological conditions like psoriasis, chronic sun-damaged skin and neoplasms: the variations of activities that are reported in these skin diseases can acquire a more substantial meaning only after we have ascertained the constancy of activity in physiological conditions.

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ADENOSINE 3' : 5'-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE IN SKIN

I. MEASUREMENT AND PROPERTIES

P. D. MIER AND E. URSELMANN

Summary.—The degradation of adenosine 3' : 5'-cyclic monophosphate (cAMP) to adenosine 5'-monophosphate by a cutaneous phosphodiesterase has been demonstrated for the first time. The ratio of phosphodiesterase : adenyl cyclase activity in skin is the same as that found in liver and brain. Certain properties of the cutaneous enzyme have been studied; in its solubility, pH curve, K_m and sensitivity to methylxanthines it seems very similar to cAMP phosphodiesterases of other tissues.

These findings suggest that the current view of cAMP as a " second messenger " in the more active tissues is equally applicable to skin.

THE importance of adenosine 3' : 5'-cyclic monophosphate (cAMP) as the " second messenger " is now well documented for many tissues. It is synthesized from ATP by the enzyme adenyl cyclase (probably identical to the " beta adrenergic receptor "), and is subsequently broken down to adenosine 5'-monophosphate by the enzyme cAMP phosphodiesterase. Thus the level of cAMP which can be attained in a cell is governed by the relative activities of these 2 enzymes. For example, the increase in intracellular cAMP resulting from hormonal stimulation of adenyl cyclase may often be mimicked by administration of an inhibitor of cAMP phosphodiesterase such as caffeine or theophylline.

Clearly, therefore, information concerning both the synthetic enzyme and the degradative enzyme is a pre-requisite to any quantitative understanding of the role of cAMP in the control mechanism of a tissue. In the case of skin, the former enzyme has already been studied (Mier and Urselmann, 1970); in this report we describe our investigation of the degradative enzyme, cAMP phosphodiesterase.

MATERIALS

Reagents were of analytic grade. The 5'-nucleotidase was a crude preparation from *Crotalus adamanteus* venom (Sigma Chemical Co.); preliminary experiments showed it to contain no enzymatic activity with cAMP as substrate; it was therefore used without further purification. Adenosine 3' : 5'-cyclic monophosphate-P^{32} was purchased from the Radioachemical Centre (Amersham, England) as the ammonium salt with a specific activity of 900 mCi/mmole.

All specimens of skin were from the backs or flanks of adult albino mice. The animals were killed by a blow to the head; after shaving, a sample of skin was removed and dissected free of subcutaneous muscle. The skin was homogenized in 0·04 mol/l tris buffer (pH 7·5) using a " micro wet grinder " (Townson and Mercer) modified by the

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inclusion of an ice-jacket. ^{32}P activity was measured with an end-window G.M. counter.

EXPERIMENTAL

(1) *Assay procedure.*—The adenosine 5′-monophosphate formed as the end-product of the cAMP phosphodiesterase reaction was further cleaved by 5′-nucleotidase to adenosine and orthophosphate; the latter was converted to phosphomolybdate, isolated by solvent distribution (method of Berenblum and Chain, as described by Lindberg and Ernster, 1954), and measured. Preliminary experiments indicated that the quantity of orthophosphate liberated was too small for reliable colorimetric assay; cAMP-^{32}P was therefore used as substrate, and the reaction followed by the appearance of ^{32}P in the organic phase.

The reaction mixture contained 0·4 μmol cAMP, 0·2 μCi cAMP-^{32}P, 2 μmol $MgSO_4$, 0·1 mg 5′-nucleotidase and 40 μmol tris in a total volume of 0·5 ml (pH 7·5). The reaction was initiated by the addition of 0·5 ml homogenate in 0·04 mol/l tris, and the mixture incubated at 37 °C. After an appropriate interval (usually 30 min) the reaction was terminated by the addition of 0·5 ml of 20% aqueous trichloroacetic acid. The supernatant was decanted into stoppered test-tubes, 0·2 ml 5 mol/l H_2SO_4, 1·0 ml benzene-isobutanol (1 : 1) and 0·2 ml 10% ammonium molybdate added, and the tubes shaken vigorously for 15 s. Aliquots of both upper and lower layers were transferred to planchettes, dried, and the ^{32}P activity measured. cAMP phosphodiesterase activity was calculated as mμmol of orthophosphate liberated per min per g of skin.

Control experiments omitting the incubation period showed that the orthophosphate content of the cAMP-^{32}P preparation was very low (0·21%); this value remained constant during the whole period of the investigation. Blanks in which the homogenate was replaced by 0·04 mol/l tris showed zero net synthesis of orthophosphate. In the following experiments the total orthophosphate was therefore corrected for the orthophosphate content of the cAMP to obtain the net enzymic synthesis; routine blanks were not run with each assay.

The level of cAMP phosphodiesterase activity found in mouse skin was low but consistent; samples from 10 different animals gave a mean value of 61 mμmol/min/g with a standard deviation of 10·7 mμmol/min/g. In order to obtain a direct comparison with the cAMP phosphodiesterase levels of other tissues of the same animal, an experiment was carried out in which homogenates of brain (0·5 mg/ml), heart (2 mg/ml), liver (2 mg/ml) and lung (2 mg/ml) were also assayed; the measured activities were 3,500, 450, 420 and 340 mμmol/min/g respectively.

(2) *Linearity with time and enzyme concentration.*—A reaction mixture was prepared at the usual concentrations but of a total volume of 5 ml. The reaction was initiated by the addition of 5 ml homogenate (20 mg/ml), and aliquots of 1·0 ml removed at 10 min intervals during a total period of 60 min. The assay of each sample was completed in the usual manner. In a second experiment, an homogenate was prepared at 50 mg/ml, and dilutions made in the range 10–50 mg/ml assays were carried out as before, with an incubation period of 30 min.

The net synthesis of orthophosphate was found to bear a linear relationship with respect both to time and to the concentration of enzyme over the whole of the ranges examined.

(3) *Solubility.*—Homogenates (30 mg/ml) from the skins of 2 animals were centrifuged; the residues were washed twice with a large excess of 0·04 mol/l

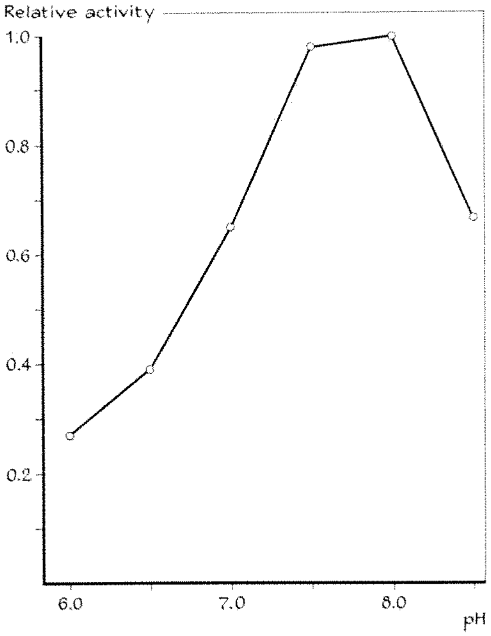

FIG. 1.—Curve illustrating the variation of mouse skin cAMP phosphodiesterase activity with pH.

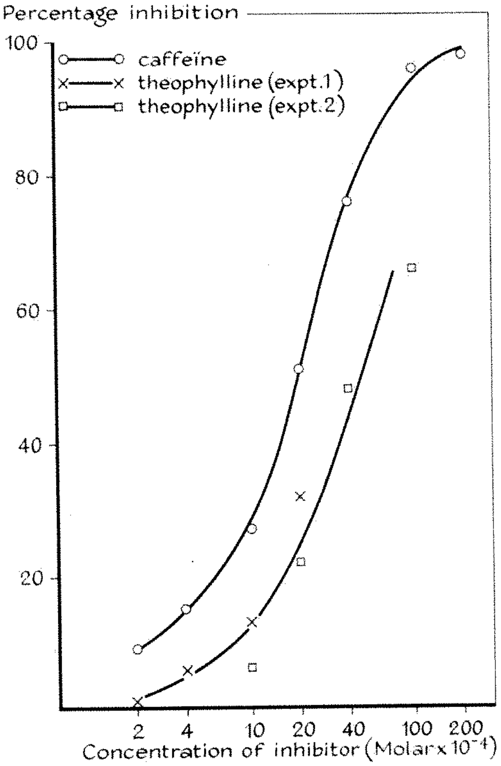

FIG. 2.—Inhibition of mouse skin cAMP phosphodiesterase by methylxanthines.

tris buffer and resuspended in buffer at the original concentration. Assays were conducted on aliquots of the whole homogenates, the supernatants, and on the re-suspended particulate fractions.

The results are shown in Table I; it is seen that substantial enzyme activity is present in both soluble and particulate fractions.

TABLE I.—*Soluble and Particulate cAMP Phosphodiesterase Activity in Mouse Skin*

Animal no.	Activity (mμmol/min/g) Whole skin	Soluble	Particulate	% age recovery
1	52	34	18	100
2	46	24	18	91

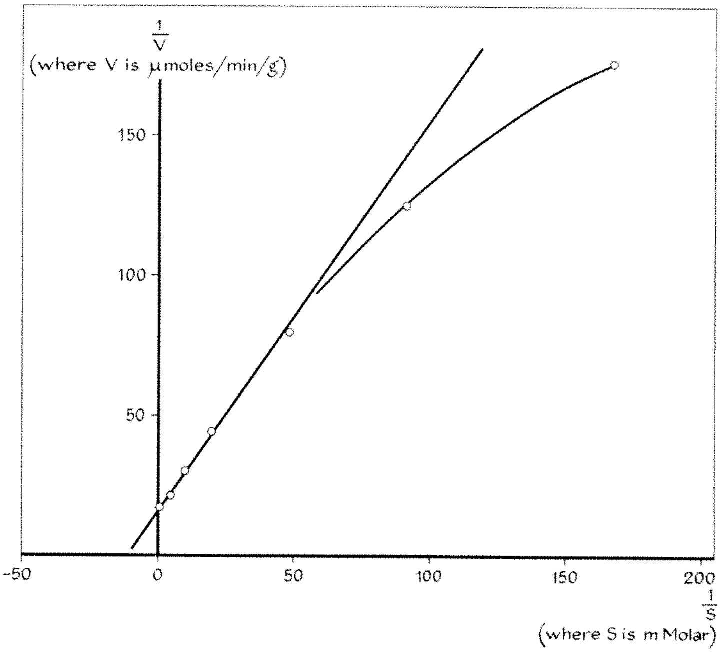

FIG. 3.—Variation of mouse skin cAMP phosphodiesterase activity with substrate concentration. A double reciprocal (Lineweaver-Burke) plot is employed; extrapolation to the 1/S axis yields a K_m (Michaelis-Menten constant) of 9×10^{-5} mol/l for the enzyme.

(4) *Effect of pH.*—Portions of the reaction mixture were adjusted to pH values in the range pH 6·0–8·5. A skin homogenate was prepared at 30 mg/ml in distilled water, and a series of assays carried out as before.

The resulting pH activity curve is illustrated in Fig. 1; an arbitrary value of 1·0 has been assigned to the activity at pH 8·0.

(5) *Inhibitor studies.*—The effects of caffeine (1 : 3 : 7-trimethylxanthine) and of theophylline (1 : 3-dimethylxanthine) on the activity of cutaneous cAMP phosphodiesterase were investigated. Aqueous solutions of the inhibitors were prepared at 0·2 mol/l and 0·1 mol/l respectively, and dilutions made to 2×10^{-3} mol/l in each case. Assays were carried out in which 0·1 ml water was replaced by 0·1 ml of inhibitor solution at the appropriate strength; the homogenate

was 30 mg/ml and the incubation period 30 min. In order to obtain a direct comparison with other tissues of the mouse, an additional experiment was carried out in which theophylline at a final concentration of 10^{-3} mol/l was tested against homogenates of brain, heart, liver and lung.

The results are shown in Fig. 2. It is seen that both of the methylxanthines inhibit the cutaneous enzyme; caffeine is somewhat more potent than theophylline (50% inhibition at $1{\cdot}9 \times 10^{-3}$ mol/l and $4{\cdot}8 \times 10^{-3}$ mol/l, respectively). The effects of 10^{-3} mol/l theophylline on the cAMP phosphodiesterase activity of other mouse tissues were of the same order of magnitude as on the skin enzyme; brain, heart, liver and lung homogenates showed 36%, 8%, 21% and 32% inhibition respectively.

(6) *Effects of substrate concentration.*—A series of assays was carried out in which the final concentration of cAMP was varied over the range 10^{-3} mol/l to 5×10^{-6} mol/l; the skin homogenate was 30 mg/ml and the incubation period 30 min.

The results are illustrated in Fig. 3 as a graph of the reciprocal of the reaction velocity against the reciprocal of the substrate concentration (Lineweaver-Burke plot). It is seen that this plot is linear above a substrate concentration of about 2×10^{-5} mol/l; extrapolation yields an apparent K_m of 9×10^{-5} mol/l.

DISCUSSION

The presence of cAMP phosphodiesterase has been demonstrated in skin for the first time. As in the case of adenyl cyclase, the very low levels of activity in the tissue necessitated the use of a radioisotopic technique. The method employed here proved highly satisfactory; the blanks were low and constant, the orthophosphate synthesis was linear with both time and enzyme concentration over a wide range, and the sensitivity was very much greater than that possible using chemical techniques.

In terms of absolute activities, our figures for the cAMP phosphodiesterase activity of various tissues appear to be lower than those of Sutherland (1961) by a factor of about 10 fold; the reason for this is uncertain. It is clear, however, that the cAMP phosphodiesterase level is always much higher than that of adenyl cyclase; the ratios of the 2 enzymes have been calculated for brain, liver and skin (Table II). In terms of the current model, a relatively high level of the degradative enzyme is theoretically necessary to enable a cell to achieve a rapid transient response to hormone stimulation, i.e. to " switch off " as soon as the stimulus is removed. Thus the striking similarity in the ratio cyclase : phosphodiesterase between skin and the better known tissues is strong circumstantial evidence that the function of cAMP in skin is also that of " second messenger ".

In its properties, cutaneous cAMP phosphodiesterase is very similar to preparations which have been described from other tissues. The distribution of activity between the soluble and particulate fractions, for example, is a well known feature of this enzyme. The pH curve (Fig. 1) is the same as that reported for beef heart (Sutherland, 1961); it is of interest that the curve is also identical to that of cutaneous adenyl cyclase (Mier and Urselmann, 1970), with the consequence that the cyclase : phosphodiesterase ratio is independent of pH. Inhibition by the methylxanthines is another well-known property of cAMP phosphodiesterase; this was demonstrated for the cutaneous enzyme (Fig. 2). A surprising finding was that our preparation appears to be more sensitive to caffeine than to theophylline, whereas in general the reverse holds true (Sutherland, 1961; Smith and Mills, 1970). A parallel study using homogenates of other mouse tissues indicates that this is a characteristic of the mouse rather than specific feature of skin.

The kinetics of cAMP phosphodiesterase are complex. In many mammalian tissues the Lineweaver-Burke plot is biphasic, suggesting the presence of 2 closely related enzymes with different affinities for cAMP. K_m values ranging from 10^{-3} mol/l to 2×10^{-5} mol/l have been reported for the " low affinity " enzyme, and values of about 8×10^{-7} mol/l for the " high affinity " enzyme (Somerville *et al.*, 1970; Skidmore and Hinley, 1970). Thus our value of 9×10^{-5} mol/l for the apparent K_m of mouse skin (established over the range 20–1000 μmol/l substrate concentration) is quite acceptable for a low affinity phosphodiesterase; the presence of an additional high affinity component is indicated by the non-linearity of the Lineweaver-Burke plot below this substrate concentration (Fig. 3).

TABLE II.—*Ratios of cAMP Phosphodiesterase Activity to Adenyl Cyclase Activity for Brain, Liver and Skin. The Values for Adenyl Cyclase are from Mier and Urselmann* (1970)

	Activity (mμmol/min/g)		
Tissue	cAMP phosphodiesterase	Adenyl cyclase	Ratio
Brain	3500	40	87
Liver	420	3·7	114
Skin	61	0·92	66

In summary, then, we have shown that skin possesses an enzyme system for the degradation of cAMP which is qualitatively and quantitatively similar to those of other tissues. Together with our previous demonstration of an active cutaneous adenyl cyclase, we are forced to conclude that the function of cAMP must again be that of " second messenger ". Clearly two tasks remain; first to determine the nature of the stimulus which elicits the cAMP-mediated response in skin, and secondly to establish which metabolic pathways are under the control of this ubiquitous substance.

Note added in proof.

The role of cAMP as "second messenger" in skin has now been demonstrated directly by Brønstad *et al.* (*Nature New Biology*, **223**, 79). These workers showed that the cAMP content of intact rat skin increases about 20-fold following incubation with adrenaline; the hormone sensitivity of cutaneous adenyl cyclase is apparently lost as a consequence of homogenization.

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ADENOSINE 3′:5′-CYCLIC MONOPHOSPHATE PHOSPHODIESTERASE IN SKIN

II. LEVELS IN ATOPIC DERMATITIS

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W. J. B. M. v.D. STAAK, E. URSELMANN AND J. A. WARNDORFF

Summary.—The activity of cAMP phosphodiesterase under conditions of substrate saturation (V_{max}) and its substrate affinity (K_m) were measured in biopsy specimens from the lesions of atopic dermatitis, from atopic uninvolved skin, and from the skin of healthy controls. No significant difference was found between the atopic and the control specimens.

The " beta-adrenergic " theory of atopy, proposed by Szentivanyi (1968), suggests that most of the clinical and experimental observations about this condition may be explained on the basis of a diminished beta-adrenergic response. Obviously this is a most attractive idea, since it offers the possibility of interpreting a great deal of apparently unrelated phenomena in terms of a single inborn metabolic error. In his original hypothesis Szentivanyi assumed this metabolic error to be simply a deficiency in the activity of the beta-receptor (the enzyme adenyl cyclase); however, attempts to demonstrate such a deficiency in the skin of atopic patients were not successful (Mier and Urselmann, 1970).

It is now becoming clear that there are a number of different enzymes which are specifically concerned with cAMP-mediated control processes; thus it is possible to visualize several different genetic abnormalities which could, in theory, result in a diminished beta-adrenergic response. An obvious suspect is the enzyme responsible for the degradation of cAMP, adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase. Clearly, if this were over-produced, or had an atypical substrate affinity, the effect could be similar to that of a reduced level of adenyl cyclase activity. This idea is particularly plausible because theophylline, a specific inhibitor of cAMP phosphodiesterase, is of therapeutic value in the symptomatic relief of asthma.

We have therefore investigated cAMP phosphodiesterase in skin biopsies from healthy subjects and from patients with atopic dermatitis. Because of the high sensitivity of the present technique, it proved possible to measure the activity of the enzyme over a range of cAMP concentrations in each individual biopsy. From this data we have established values for both the enzyme activity under " saturation " conditions (V_{max}) and its substrate affinity (K_m) in normal and atopic skin. The results are presented in the following report.

MATERIALS AND METHODS

All reagents were as described previously (Mier and Urselmann, 1971). Patients were selected (" atopic ") who had a history of chronic eczematous skin lesions and who

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showed multiple positive reactions to intradermal allergen tests. Control subjects ("non-atopic") were 4 healthy volunteers who had no personal or family history of atopic illness; 2 of these were totally negative to allergen tests, and 2 showed a positive reaction to a single allergen only. Either 1 or 2 biopsies were taken from each subject, using a 4 mm punch after freezing the skin with ethyl chloride spray. All assays were started within 10 min of the biopsy being taken.

cAMP phosphodiesterase activity was measured as described previously (Mier and Urselmann, 1971). The whole biopsy was homogenized in 2·5 ml 0·04 mol/l tris (pH 7·5), and 0·5 ml aliquots of the homogenate were used to measure the reaction velocity at cAMP concentrations of approximately 20, 50, 100 and 200 μmol/l. The reciprocal of the reaction velocity was plotted against the reciprocal of the substrate concentration (Lineweaver-Burke plot), and the values of V_{max} and K_m obtained by extrapolation.

RESULTS

The Lineweaver-Burke plots were, within experimental error, linear over the range 20–200 μmol/l cAMP. The data obtained from 8 atopic and 8 non-atopic biopsies are shown in full in Table I.

TABLE I.—*Values of V_{max} and K_m of cAMP Phosphodiesterase from Individual Biopsies*

Clinical assessment	Subject No.	 Sex	 Age	Site and nature of biopsy	V_{max} (mμmol/min/g)	K_m (μmol/l)
Atopic	1	M	19	Chest (lesion)	52	110
				Upper arm (uninvolved)	21	38
	2	M	20	Upper leg (lesion)	21	42
	3	F	22	Centre back (lesion)	37	63
				Lower back (uninvolved)	29	130
	4	F	27	Upper arm (uninvolved)	13	45
	5	F	18	Upper back (uninvolved)	28	121
	6	F	17	Upper back (lesion)	22	72
Non-atopic	1	M	38	Upper arm	10	38
				Upper back	13	43
	2	M	22	Upper arm	7	45
				Upper back	16	66
	3	M	22	Upper arm	28	90
				Upper back	31	69
	4	M	24	Upper arm	15	51
				Upper back	24	128

DISCUSSION

The mean values and standard deviations for V_{max} and K_m of cAMP phosphodiesterase from atopic lesions, atopic uninvolved and non-atopic specimens are shown in Table II (columns 1 and 2). The value of V_{max} for the atopic lesion must be corrected to allow for the increased cellularity; this may be done either on the basis of the DNA content (Mier and Urselmann, 1970), or more simply by calculating the ratios of phosphodiesterase to adenyl cyclase activity (Table II, column 4). The K_m, of course, requires no correction, since it is an inherent property of the enzyme and is independent of its concentration. The differences between the atopic and the non-atopic groups are not statistically significant for either K_m or the phosphodiesterase : adenyl cyclase ratio.

An unrelated but puzzling feature of these results is the very wide scatter in the values for K_m between different specimens of skin. The scatter (38–130 μmol/l) is very much greater than can be accounted for by the experimental error ($\pm$ 20%).

TABLE II.—*Mean Value for $V_{\max}$ (col. 1) and K_m (col. 2) of cAMP Phosphodiesterase from the Atopic Lesion, Atopic Uninvolved and Control Specimens of Skin. The Corresponding Figures for Adenyl Cyclase (Mier and Urselmann,* 1970) *are Shown in col.* 3, *and the Ratios in col.* 4

	cAMP phosphodiesterase			
	$V_{\max}$ (mμmol/min/g)	K_m (μm)	Adenyl cyclase (mμmol/min/g)	Ratio phosphodiesterase: adenyl cyclase
Atopic lesion (4)	33±13	72±25	1·05	30
Atopic uninvolved (4)	23±6	84±42	0·59	39
Non-atopic (8)	18±8	66±28	0·46	39

The only variable with which a significant correlation can be made is that of the site of biopsy; the mean K_m of cAMP phosphodiesterase of skin taken from the limbs is 50 μmol/l, whereas that from the trunk is 89 μmol/l. This aspect of the investigation is being extended and will be the subject of a separate communication.

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THE CUTANEOUS REACTIONS PRODUCED BY *o*-CHLOROBENZYLIDENEMALONONITRILE AND *ω*-CHLOROACETOPHENONE WHEN APPLIED DIRECTLY TO THE SKIN OF HUMAN SUBJECTS

P. HOLLAND AND R. G. WHITE

Summary.—The cutaneous reactions produced by the irritant compounds *o*-chlorobenzylidenemalononitrile, and ω-chloroacetophenone, have been studied on human subjects. ω-Chloroacetophenone has been shown to present a far greater cutaneous hazard than *o*-chlorobenzylidenemalononitrile when applied directly to the skin. The cutaneous reactions produced are worse when the irritant is moistened.

o-CHLOROBENZYLIDENEMALONONITRILE has, because of its high safety factors, replaced *ω*-chloroacetophenone for riot control purposes (Finn *et al.*, 1960). When dispersed as an aerosol it produces irritation of the eyes and upper respiratory tract at concentrations as low as 0·1 mg/m^{-3}, over periods of exposure as short as 1 min. These symptoms disappear rapidly when the exposed subjects move to fresh air (Punte *et al.*, 1963).

Because of the nature and rapidity of its action, those exposed to it, as an aerosol, are highly motivated to escape from the cloud, thus limiting the possibility of over-dosage and therefore the chances of initiating skin reactions. Only in exceptionally severe conditions of exposure are cutaneous reactions likely to be expected; thus, experimentally, cutaneous reactions can be produced when the crystalline material is placed in contact with the skin, or when high doses of aerosol are passed over the skin under somewhat unrealistic conditions. Weigand (1969) describes experiments in which the forearms of volunteers were exposed to a very high concentration of *o*-chlorobenzylidenemalononitrile, greater than 14,000 mg/m^{-3}, for 1 hour under simulated tropical conditions; extreme irritation, erythema and vesication were produced. He also stated that the cutaneous reactions observed showed wide individual variation, depending on climatic conditions, race and skin characteristics.

The cutaneous effects of *ω*-chloroacetophenone have not been so thoroughly investigated but, like *o*-chlorobenzylidenemalononitrile, *ω*-chloroacetophenone aerosol (and vapour) is a potent irritant, qualitatively producing similar effects on the eyes and skin. On the skin the severity of reactions varies according to the concentration of irritant, the state of the skin, i.e. whether it is moist or dry, and the duration of contact. Vedder (1925) found that *ω*-chloroacetophenone vapour had very little effect on the skin of man but, when applied in excessive amounts, it could produce erythema and vesication. Several authors (Kissen, 1964; Madden, 1951; Queen, 1941) describe cutaneous reactions occurring after single and multiple exposures to its vapour. Ingram (1942) reported that irritation, erythema and

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TABLE I.—*Numbers of Individuals with Reactions Produced by o-Chlorobenzylidenemalononitrile and ω-Chloroacetophenone When Applied to the Skin of the Forearm*

	mg	0·5	1·0	2·0	5·0	10·0	15·0	20·0	25·0	30·0
o-Chlorobenzylidenemalononitrile	Dry	—	—	—0/6	—0/6	—0/6	—0/6	I± 3/3 e±	I± 3/7 e±	—
ω-Chloroacetophenone		I+ 4/6 e+ 6/6 v—	I± 6/6 e± 6/6 v—	I++ 5/6 e++ 6/6 v++ 3/6	—	—	—	—	I++ 4/6 e++ 5/6 v++ 1/6	—
o-Chlorobenzylidenemalononitrile	Wet	—	—	—	—	I± 4/4 e± 4/4	—	I+ 4/4 e+ 4/4	I+ 3/7 e+ 3/7	I+ 4/4 e+ 3/4
ω-Chloroacetophenone		I± 6/6 e++ 6/6 v++ 4/6	I++ 6/6 e++ 6/6 v++ 6/6	I++ 6/6 e++ 6/6 v++ 6/6	—	—	—	—	I+++ 6/6 e+++ 6/6 v+++ 6/6	—

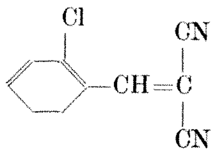
Key: I, irritation; e, erythema; v, vesication; — negative; ±, slight; +, mild; ++, moderate; +++, severe.

blistering can occur following exposure to high concentrations in confined spaces. Thus both these compounds in the aerosol form are capable of producing cutaneous reactions in conditions which are far removed from the usual practical anti-riot circumstances.

This report extends previous observations to include the effects of both *o*-chlorobenzylidenemalononitrile and *ω*-chloroacetophenone when applied directly to the skin. No attempt has been made to study the sensitizing potential, if any, of these compounds following repeated applications.

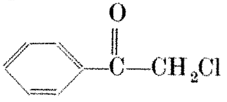
MATERIALS

o-Chlorobenzylidenemalononitrile



Recrystallized × 2 from alcohol.
Pure.
White crystals.

ω-Chloroacetophenone



Recrystallized × 1 from alcohol.
95% purity.
White crystals.

PROCEDURE

The subjects were healthy volunteer servicemen, aged between 18 and 30, with no history or clinical evidence of skin disease or allergy. Each compound was applied in graded quantities (Table I) to the skin of the forearm, under a watch glass (4 cm diameter) kept in place with Sleek dressing. Where possible, subjects were investigated in groups of 6, each group at a different dose level. The materials were applied (a) dry and (b) moistened with 2 drops of saline. The duration of contact was 1 hour, the substance then being removed with a dry swab, followed by washing with soap and water. If a volunteer complained of any undue discomfort at less than 60 min, the experiment was immediately terminated. The test site was examined at 1 hour, 6 hours and subsequently daily, for the appearance and development of skin lesions.

RESULTS

Table I summarizes the incidence of cutaneous reactions produced by varying amounts of *o*-chlorobenzylidenemalononitrile and *ω*-chloroacetophenone, the number of subjects involved and the quantities applied.

o-Chlorobenzylidenemalononitrile

The reactions produced were slightly more severe when the material was moistened. In the dry state, over the range 2·0 mg to 30 mg, only at 20 mg was there a faint erythema and transient irritation commencing up to 30 min following application. When moistened, only quantities of 10·0 mg and over produced mild irritation within 30 min, which disappeared following its removal. Faint erythema was observed following application of quantities over 10·0 mg, when moist (Fig. 1). Depending on the amount applied, it remained (fading gradually) for anything up to 1 to 2 days, leaving no residual pigmentation or blanching of the skin. No irritation or pruritus was complained of during the stage of erythema. No vesication occurred.

ω-Chloroacetophenone

The cutaneous reactions produced by this substance were more severe when it was applied moist. Amounts of 0·5 mg produced irritation and erythema and, when moistened, vesication in more than 50% subjects. Irritation, experienced within 10 min by all subjects, and at all dose levels, became more severe during the period of application, and at 25·0 mg it was so severe that the skin had to be decontaminated within 30 min. The irritation disappeared following removal of the material, but recurred transiently when washing the affected area during the subsequent 12 hours.

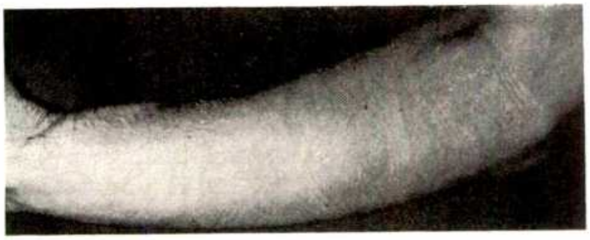


FIG. 1.—Erythema produced by moistened *o*-chlorobenzylidenemalononitrile. Reading L → R, 10·0 mg; 20·0 mg; 30·0 mg.

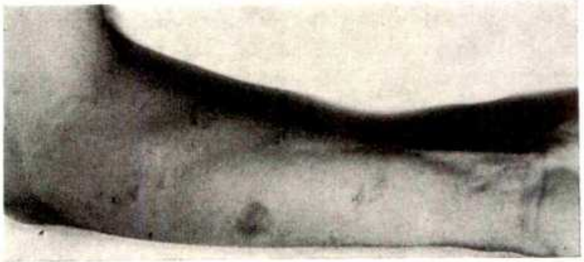


FIG. 2.—Vesicles produced by moistened ω-chloroacetophenone. Reading L → R 2·0 mg; 1·0 mg; 0·5 mg.

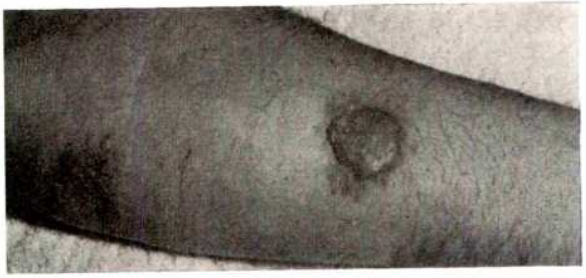


FIG. 3.—Vesicle produced by 25·0 mg ω-chloroacetophenone (moistened).

In every instance erythema was diffuse (× 3 the original area of application) and appeared within 30 min. At doses over 2·0 mg there was also, in the majority, localized oedema, which had subsided after 24 hours, while the erythema was becoming localized to the original application site. When quantities of 0·5 mg to 2·0 mg were applied dry, the erythema produced faded quickly and disappeared by 72 hours. At all other dose levels, and with wet applications below 2·0 mg, the erythema subsequently became fully localized, well demarcated, raised and papular. The papules later coalesced to form a ring of vesicles at about 48 hours (Fig. 2). Small vesicles were allowed to resolve spontaneously over the ensuing 3–4 days, but a few larger vesicles of about 1 cm diameter (Fig. 3) were aspirated to promote healing and minimize accidental bursting and possible infection. This healing process took up to 1 week, during which the blistered areas were kept covered with dry sterile gauze dressings.

Two weeks later the lesions were evident as faint areas of pigmentation. A follow-up of these subjects, arranged with the unit medical officers, confirmed that healing was complete, with no residual scarring or skin discolouration.

DISCUSSION

Under comparable conditions, equivalent doses of *o*-chlorobenzylidenemalononitrile and ω-chloroacetophenone produce effects on skin such that those due to the former are much less severe than those produced by the latter.

Ingram (1942) describes the case of an officer who was exposed to a " fairly high concentration of ω-chloroacetophenone (CAP) in a confined space—for three-quarters of an hour ". Over 7 hours later this man developed blisters, but there were no other symptoms, and his general health was not affected. Weigand (1969) showed that dosages of *o*-chlorobenzylidenemalononitrile of 10 gm/min/m^3, which are probably much higher than those of CAP in the Ingram report, produce only erythema, and dosages of the order of 14 gm/min/m^3 are required, in moist warm conditions, to produce vesication some 12 to 24 hours later.

Treatment was not considered necessary in the subjects of this investigation; but, in the event of extreme irritation, the use of calamine cream, with antihistamine therapy at night to aid sleep, is recommended. Blisters resolve spontaneously, but larger ones may be aspirated and the raw areas kept clean with dry sterile gauze dressings. Antibiotics may be used to control infection.

This material was reported to the Committee of Enquiry into the *Medical and Toxicological Aspects of CS* and is referred to in Part II of the Report (HMSO Command Paper 4775) by that Committee as Reference No. 25, Committee Report 6290/70.

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Sabah Hospital, Kuwait

CEMENT DERMATITIS AND ATMOSPHERIC PARAMETERS IN KUWAIT*

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Summary.—A series of 191 male patients, 2 Kuwaitis and 189 non-Kuwaiti immigrants from the Middle East, with cement dermatitis, is reported. On the first visit 108 patients had already passed into the chronic stage and 40% showed an irregular nummular pattern. On patch testing, all 191 patients reacted positively to 0·5% potassium dichromate but only 13 of them reacted to 5% cobalt chloride, which incriminated the chromates as the significant allergen in cement dermatitis in Kuwait. Of the 191 patients, only 16 had suffered from transient dermatitis in their countries of origin. Seventy-seven of the remaining 175 patients came in contact with cement for the first time in Kuwait, whereas the other 88 patients had never had dermatitis in their countries of origin, in spite of long contact with cement. Of 112 patients who were followed up for a period longer than 2 years, 84 (75%) were observed to have clinical exacerbation between April and mid-November each year. The curve describing the latter was found to be a direct function of the curve obtained for the temperature monthly means. The rising humidity during September each year overtook the high temperature, which probably provoked excessive perspiration and thus favoured the leaching of chromate ions by the integument.

THE swift urbanization since the recent discovery of oil has turned Kuwait into a concrete jungle. Consequently cement dermatitis has become a pressing occupational problem. This paper presents a study of cement dermatitis undertaken during the period April 1st, 1966 to January 1st, 1970.

MATERIALS AND METHODS

This investigation comprised 209 patients recruited by colleagues in the Skin Clinic, Amiri Hospital, Kuwait. A definite clinical diagnosis of cement dermatitis could be made in only 191 patients. Every patient was fully interrogated about his complaint, his occupation, the duration of the disease and exacerbations. The lesions were fully examined and, whenever possible, were photographed. All referred patients were patch tested during the quiescent phase with potassium dichromate 0·5% in petrolatum, cobalt chloride 5% in petrolatum and physiological saline as a control. Testing with trivalent chromium compounds was not performed as suitable material was not available. Readings were recorded after 48 hours and after 96 hours. Patients were not allowed any systemic antihistamines or corticosteroids until after the second reading had been made. All patients were followed up, and detailed records were kept for further analysis. The frequency of their attendance at the clinic varied with the severity of their symptoms.

The monthly means of atmospheric temperature and relative humidity were copied from the meteorological office records (Statistical Abstract, 1970).

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* Based on a paper presented at the American University of Beirut, May 1971.

Requests for reprints should be addressed to Dr Kanan, P.O. Box 6050, HL, Kuwait, Arabia.

RESULTS

The 191 patients who were found to have cement dermatitis were adult males whose ages ranged between 18 and 60 years. Two of them only were Kuwaitis; the others came from neighbouring Middle East countries. Various occupations entailing contact with cement were encountered. Wall plastering was recorded in 105 cases, floor tiling in 33, ordinary labour in 21, building in 12, pipe handling in the asbestos factory in 8, bricklaying in 5, carpentry in 5, and plumbing in 2 cases. The total time spent in the occupation varied from 1 month to 35 years. Only 16 patients suffered from mild dermatitis in their country of origin before arriving in Kuwait. Of the remaining 175 patients who developed cement dermatitis after arrival in Kuwait, 88 patients (51 %) had never had dermatitis in their country of origin, in spite of long contact with cement. The remainder (87 patients) came into contact with cement for the first time after their arrival in Kuwait. Of those who developed the dermatitis in Kuwait 3 had it after only 4 weeks of contact with cement. Eighty-four others had it after 1 month to 1 year,

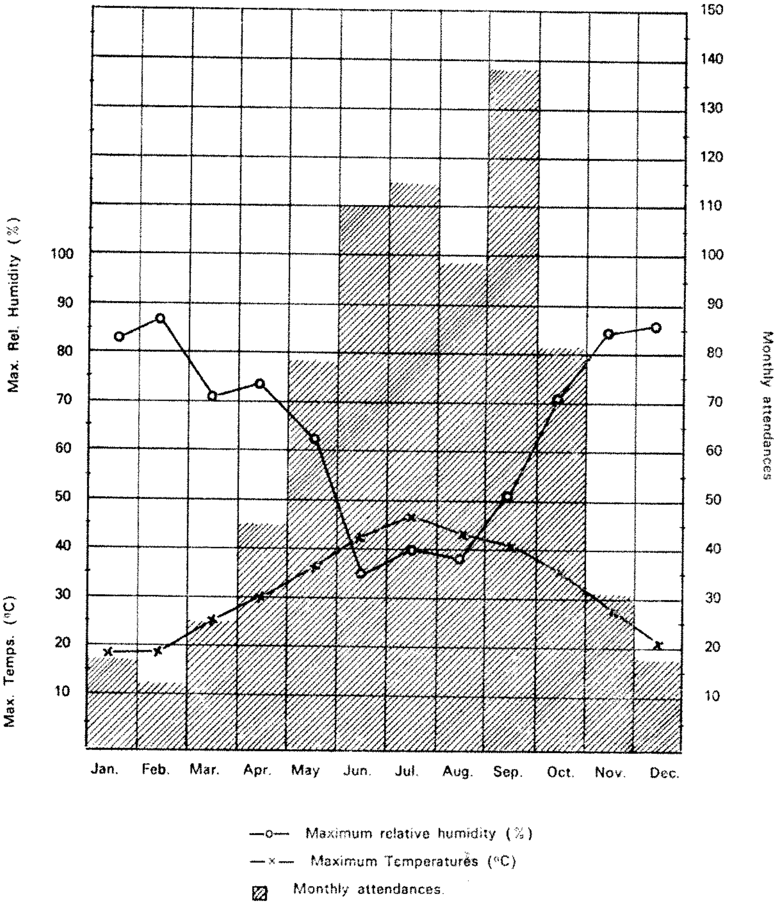

FIG. 1.—Monthly means for 1968.

71 after 1 to 5 years, and the remainder after 5–20 years' contact. Eighty-four of 112 patients (75 %) who had the disease longer than 2 years were observed to have clinical exacerbations only during the period from April to mid-November each year, and were almost symptomless in the remaining months of the year. The other 28 patients had the dermatitis through the whole year, although 10 of them felt better during the cool winter months. It is quite clear from Fig. 1 that the total monthly attendances of patients with cement dermatitis rose with a concordant rise in the atmospheric temperature during 1968. Our statistics for the other years of the investigation (obtainable from the author) show the same clear relationship between temperature and the activity of the dermatitis. Regularly, September records showed maximum monthly patient attendances, while the minimum monthly attendances were recorded during December, January and February. The incidence rose in mid-March and declined in mid-October each year.

Clinical picture

The commonest clinical pattern was seen in a group of 78 patients who presented with sub-acute or chronic nummular eczema on the upper and lower limbs. The nummular patches differed from those of the constitutional type of eczema by their irregular outline and asymmetrical distribution. A second group of 54 patients showed acute papulo-erythematous dermatitis, with or without vesiculation. A third group of 30 patients presented with a picture of chronic, diffuse, lichenified dermatitis, while the fourth clinical picture was encountered in a group of 29 patients in the form of vesiculo-pustular eczema. The most commonly involved sites were the backs of the hands and fingers, the flexor aspects of the forearms and the back of the feet. The results of skin patch testing are shown in Table I.

TABLE I.—*Cement Dermatitis Results of Patch Testing*

		½% Pot. dichrom.		5% Cob. chloride	
Patients	No.	Positive	Negative	Positive	Negative
Cement dermatitis cases	191	191	—	13	178
Other hand dermatitis cases	18	—	18	—	18
Healthy workers in asbestos	81	2	79	—	81

Follow-up and prognosis

The majority of the patients (108 cases) had already passed into the chronic stage of dermatitis when they first presented. It was impossible to assess the effect on the course of their dermatitis, of moving patients from their skilled jobs, because of the high wages they earned. Three of some 21 patients with acute cement dermatitis who were persuaded to leave their jobs continued to suffer from chronic chromate dermatitis in spite of lack of any further contact with cement or any other evident source of chromates. The other 18 patients were lost after their dermatitis had healed for further follow-up, either because they were cured or because they had left the country. The majority of the patients on further follow-up to the time of writing were labelled as cases of chronic recurrent cement dermatitis.

DISCUSSION

The very low incidence of cement dermatitis in Kuwaitis (only 2 cases) has certainly been attributed to both social and financial factors. The Kuwaiti youth on the whole do not indulge in manual labour. Skilled labourers in the building trade are tempted to come from other countries of the Middle East to work in Kuwait because of higher wages. However, Kuwaitis are as prone to chromate dermatitis as other nationalities. This was confirmed in a simultaneous study of footwear dermatitis (Kanan, 1969). It was quite remarkable to learn that 88 of 104 patients (84 %) who had contacted cement for many years in their countries of origin had never suffered from cement dermatitis before they started working in Kuwait. The remaining 16 patients gave a history of ephemeral bouts of mild dermatitis in their countries of origin, healing fairly well with treatment. This phenomenon could not be attributed to a higher chromate content in the cement used in Kuwait. The latter is not of one make and is still being imported from various countries of the world. All 191 patients in the present series yielded positive reactions to 0·5 % potassium dichromate, and only 13 of them reacted positively to cobalt chloride. This finding seems to incriminate hexavalent chromates as a very significant offender, while it probably excludes cobalt as an effective cement allergen in this country.

Hunziker and Musso (1960) found that a high percentage (51 %) of their cement dermatitis cases had a positive reaction to cobalt. Geiser *et al.* (1960) also found that 51 % (27 cases) of their cement dermatitis patients had reacted positively to 2 % cobalt chloride and 92 % (49 patients) had given positive reactions to 0·5 % potassium dichromate; whereas Pirila (1954) found 3 positive reactions to cobalt nitrate among 90 patients with cement dermatitis. However, a comparatively higher percentage of chromate positivity was obtained in the present series than in that of Burrows and Calnan (1965) who reported 78 % of their patients chromate positive; only one of these was sensitive to 5 % cobalt chloride. Nevertheless the very high correlation of clinical and patch test diagnosis can be attributed partly to the scarcity of endogenous eczema in our material.

In a personal series of 81 healthy workers recruited from the Kuwait asbestos factory only 2 yielded positive reactions to 0·5 % potassium dichromate. One of them gave a history of leather sandal dermatitis 2 years previously. Burrows and Calnan (1965) expressed the opinion that too much reliance should not be placed on the patch test in the diagnosis of cement dermatitis and that it should be assessed with the history, occupation and type of eczema. The clinical exacerbation observed in 75 % of those patients followed up for longer than 2 years was reflected on the curve of monthly patients' attendances, which was almost a direct function of the curve of maximum temperature monthly means. It is also obvious from the figure that the highest incidence always followed the cross-over point of atmospheric temperature and relative humidity.

Thus the rising humidity during September each year overtook the high temperature to provoke excessive perspiration in susceptible individuals. This, in turn, helped the leaching of the chromate allergen by their integument. This assumption is supported by Samitz and Gross's work (1960), which showed that human sweat could leach chromium compounds from chrome-tanned leather in patients with leather footwear dermatitis. Hjorth (1968) reported a general low incidence of contact dermatitis in Denmark during summer, but also recorded an influx of patients referred for chromate dermatitis, due mainly to cement, in the late summer. No attempt was made to explain the latter finding or to correlate it with the local atmospheric temperature and humidity. Clinically, the high incidence of an irregular nummular pattern of dermatitis in the present series (41 %) is in agreement with impressions of other authors that this is a common feature of chromate-induced dermatitis (Hall, 1944; Walsh, 1953; Calnan, 1960; Burrows and Calnan, 1965).

In conclusion, a longer follow-up is necessary to judge the effect of early removal on some patients who agreed to leave their jobs soon after they developed their dermatitis. On the whole follow-up of our patients seems to agree with Samitz's experience (1956) that chromate dermatitis tends to become chronic, even when the patient has been removed from direct contact with chromates. Several authors have however expressed the opinion that chrome is difficult to avoid in everyday life, and traces of it can be found in paper, matches, wood ashes and even in potato peel (Calnan, 1960; Fregert, 1962; Burrows and Calnan, 1965).

I wish to express my sincere thanks to all my colleagues in the Skin Department, Amiri Hospital, Kuwait for their cooperation.

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Comparative Dermatology

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INNERVATION OF SWEAT GLANDS IN HORSES AND DOGS

MARY BELL AND WILLIAM MONTAGNA

Summary.—The sweat glands in the digital pads of dogs are surrounded by nerves that are clearly demonstrated with techniques for acetyl- and butyrylcholinesterase. The glands in the hairy skin have no such nerves. In the horse, despite some variation in the abundance of demonstrable nerves around the glands, the latter are surrounded by nerves that are demonstrated only with techniques for acetylcholinesterase.

THIS communication demonstrates cholinesterase-reactive nerves around the sweat glands in the digital pads of dogs and in the hairy skin of horses. Only Aoki (1964, 1970) has shown these nerves; but his work, although convincing, is little known, and his illustrations lack precision and clarity.

MATERIALS AND METHODS

Specimens of digital pads were obtained from 5 dogs; specimens of the nose, flank, eyelid, lip, back and interdigital skin were taken from only one of these dogs. Upper lip, flank, mane, perineum, saddle (lumbar), and chest skin was collected from 2 horses. Thirty to 100 μ-thick sections were cut in a cryostat and incubated for 5 hours at pH 5·2 in media for the demonstration of acetyl- and butyrylcholinesterase (Koelle and Friedewald, 1949) as modified by Roman *et al.* (1969). Control skin from the digital pads of dogs was incubated in histochemical media containing 10^{-5} mol/l eserine (Physostigmine salicylate).

RESULTS

The Dog

All of the glands in the digital pads of dogs were surrounded by nerves that reacted to substrates for both acetyl- and butyrylcholinesterase, but more intensely to the former (Figs. 1, 2, 3). The coils of the glands were also strongly reactive for both acetyl- and butyrylcholinesterase activity, and in some glands the reactivity was inside the lumina (Fig. 2). Both acetyl- and butyrylcholinesterase activities in the nerves were completely inhibited by 10^{-5} mol/l eserine in the incubating media, but the enzyme activity in the lumina of the glands was only partially suppressed.

The glands in the hairy skin, all apocrine, were always associated with hair follicles. We failed to demonstrate nerves in any of these glands with either

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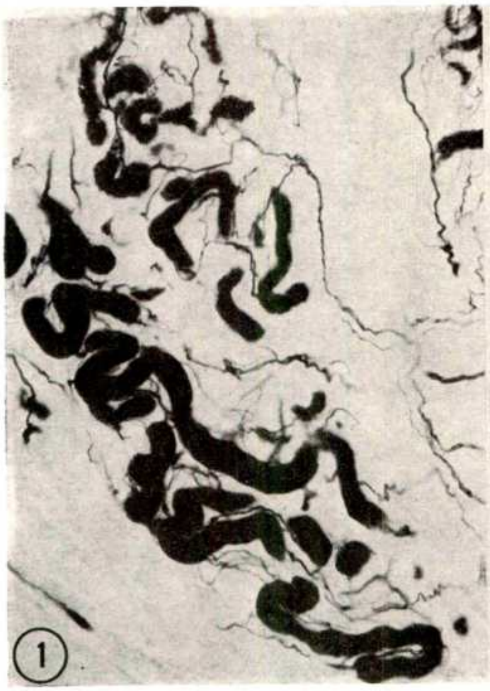
acetyl- or butyrylthiocholine iodide as substrates for cholinesterases, and none of the glandular tubules contained cholinesterase activity. The glabrous rhinarium had many cholinesterase-reactive nerves and nerve end-organs, but there were no glands in this area.

The Horse

In spite of some regional variations in the numbers of nerves, all of the glands in the hairy skin were surrounded by nerves reactive *only* for acetylcholinesterase (Fig. 4). Only an occasional gland had few or no reactive nerves around it. No enzymic activity was detected in the glandular parenchyma.



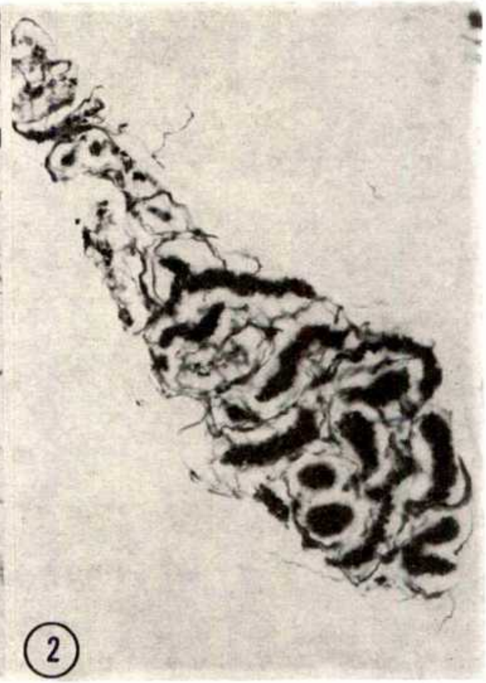


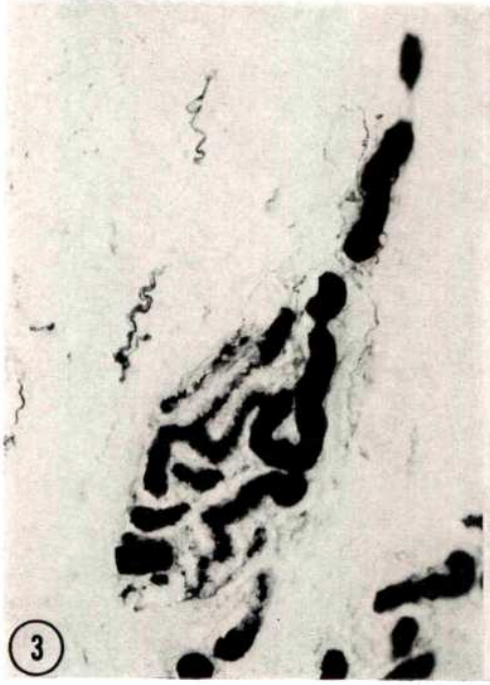
FIG. 1.—Sweat gland from the digital pad of a dog surrounded by acetylcholinesterase-reactive nerves. There is also intense reactivity in the coils of the gland. Technique of Roman *et al.* (1969). × 110.

FIG. 2.—Sweat gland in a digital pad of a dog. The tissue was prepared as in Fig. 1. There is strong acetylcholinesterase activity in nerves and in the luminal zones of secretory coils. × 110.

DISCUSSION

We have demonstrated cholinesterase-reactive nerve fibres around the sweat glands in the digital pads of the dog and the body skin of the horse. Those in the digital pads of the dog also have large quantities of enzyme in the viscus; earlier, only Aoki had demonstrated this (1964, 1966, 1970).

Several authors (Aoki, 1955, 1964; Aoki and Wada, 1951; Aoki *et al.*, 1959; Iwabuchi, 1967; Takahashi, 1964) have shown that the glands in the hairy skin of dogs and horses and those in the toe pads of dogs react to epinephrine, acetylcholine, mecholyl, pilocarpine and nicotine. The glands of the horse have only acetylcholinesterase-reactive nerves, those in the hairy skin of the dog have no nerves demonstrable with these techniques, and those in the toe pads of the dog have nerves reactive for both butyryl- and acetylcholinesterase, as well as for an enzyme or enzymes in the cells and lumen of the secretory coil. The enzyme activity in the glandular coils themselves, not inhibited by eserine, is probably a nonspecific esterase not yet identified. Since the nerves around the glands of horses are copious, the question remains as to why others have not demonstrated them and have assumed that glandular activity is mediated only by humoral stimulation (Bell and Evans, 1956). Furthermore, Aoki and Wada (1951) and Aoki *et al.* (1959) did show nerves around such glands in the hairy skin of both horses and dogs; and the glands in both animals responded to thermal stimulation yet *only* those of the horse are surrounded by cholinesterase-reactive nerves. Aoki (1964) also observed that the glands in the digital pads of dogs secrete cholinesterases in their lumina when pharmacologically stimulated. This puzzling observation suggests that the physiology of the cutaneous glandular system of these 2 animals needs to be studied systematically.



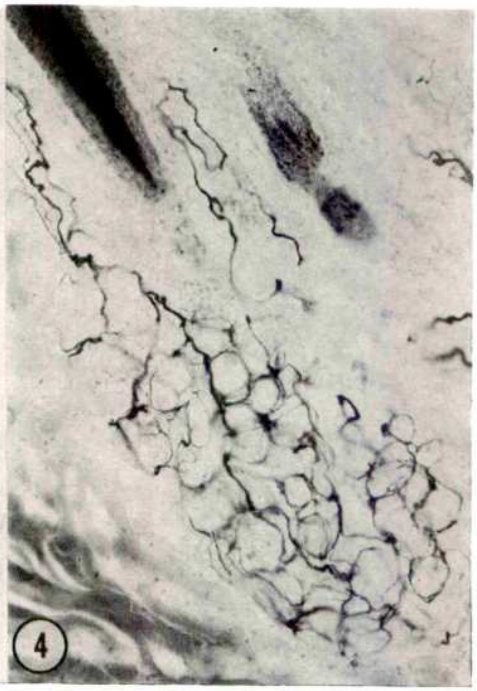


FIG. 3.—Gland in a digital pad of a dog. The tissue was prepared for the demonstration of butyrylcholinesterase. Intense activity in some glandular coils, but in others it is restricted to the luminal border. Only very few nerves are reactive. Compare with Fig. 1, 2. Technique of Roman *et al.* (1969). × 110.

FIG. 4.—Sweat gland from the flank skin of a horse showing numerous acetycholinesterase-containing nerves. Nerve fibres tightly surround the glandular coils, but the secretory cells exhibit no cholinesterase. Technique of Roman *et al.* (1969). × 110.

We wish to thank Mr Nickolas Roman for his excellent technical assistance.

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GENERALIZED CUTANEOUS ERUPTION IN A DOG, WITH HISTOLOGICAL SIMILARITY TO HUMAN MYCOSIS FUNGOIDES

D. F. KELLY*, R. E. W. HALLIWELL† AND R. M. SCHWARTZMAN

Summary.—A Boxer bitch aged 8 years had a chronic progressive cutaneous eruption of 5 months' duration. Histologically there was diffuse infiltration of the dermis, epidermis and adnexal structures by histiocytic cells. There was no evidence, at necropsy, of the presence of lymphosarcoma, mastocytoma, or histiocytoma.

The lesions are compared with those of mycosis fungoides in man.

MYCOSIS fungoides is a well-recognized nosological entity in man, characterized by infiltration of the dermis with polymorphic histiocytic or lymphoreticular cells. Secondary epidermal changes, including acanthosis, hyperkeratosis, parakeratosis, and the formation of Darier-Pautrier microabscesses, may occur in some cases. Clinically, the condition is protracted, and occasional cases may terminate as an unequivocally malignant reticulosis such as Hodgkin's disease, reticulum cell sarcoma, or lymphosarcoma. Although the association of these reticuloses with mycosis fungoides is recognized, their precise relationship to mycosis fungoides is still unresolved. Some authors incline to the view that mycosis fungoides is a clinical stage in the genesis of malignant lymphoma (Allen, 1966; Clendenning *et al.*, 1964; Evans, 1966; Robbins, 1968), but others also consider the possibility that mycosis fungoides and cutaneous malignant reticuloses are separate entities (Gould *et al.*, 1964; Marshall, 1956). Cytological examination of cells in skin windows of human patients suggests that mycosis fungoides is a non-malignant proliferation of immature reticular cells of the dermis (Vesper *et al.*, 1971).

In the dog, lymphosarcoma is among the commonest of malignant neoplasms arising outside the skin and mammary glands (Bäckgren, 1965; Dorn *et al.*, 1967, 1968a, b); but, in this species, lymphomatous involvement of the skin is relatively uncommon (Anderson *et al.*, 1969; Dorn *et al.*, 1967). Cutaneous lymphosarcoma usually occurs with co-existent lymphosarcoma elsewhere in the body. If the well-known cutaneous mastocytoma (Cook, 1969) and the canine cutaneous histiocytoma (Howard and Nielsen, 1969; Kelly, 1970; Taylor *et al.*, 1969) be excluded from consideration, lymphoreticular infiltrations of the canine skin are relatively uncommon. There is, to our knowledge, no well-documented account of a condition in dogs resembling mycosis fungoides of man.

The case presented here is of interest to comparative dermatology because of its unusual clinical features and because of some histological similarity to mycosis fungoides of man.

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CASE REPORT

Clinical history.—In August 1969, a spayed Boxer bitch aged 8 years was presented on account of a dermatological problem of 4 months' duration. There were numerous circular cutaneous lesions, essentially of 2 types: (1) raised, ulcerated plaques; (2) atrophic, erythematous, alopecic patches. There was a suggestion that some of the former lesions progressed to the latter, which sometimes arose independently. At the time of presentation approximately 1/8 of the cutaneous surface was involved, with extension to the mucocutaneous junctions. There was a tendency for the lesions to coalesce to form larger irregular areas. Hair could be pulled easily from the skin of the trunk.

Laboratory data.—There was slight haemoconcentration (packed cell volume—53%, Hb concentration—18·4 g%). The total W.B.C. was 6,700/mm^3 and the differential count (%) was: neutrophil leukocytes, 67; lymphocytes, 24; monocytes, 1; eosinophil leukocytes, 8. An LE preparation was negative. Urinalysis revealed haematuria, haemoglobinuria and proteinuria (non-quantitated). Attempts to recover fungi from the skin lesions were unsuccessful. No pathogenic bacterium was cultured from the skin. Histological examination of punch biopsies from the neck and back revealed mild oedema of the superficial dermis, and dermal accumulation of lymphocytes, plasma cells and "reticular" cells. No opinion was given on the nature of the lesion at this stage. Treatment of the dog consisted of oral administration of tetracycline and corticosteroid.

Clinical course.—Within the next month there was progressive extension of the lesions to involve approximately 25% of the surface of the skin. At the end of this month euthanasia was carried out by intravenous injection of barbiturate solution.

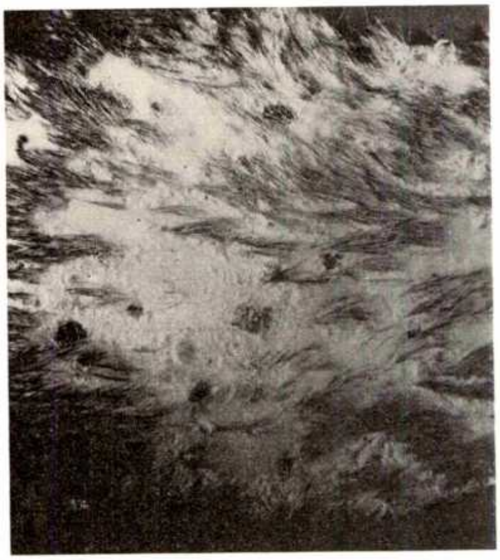
FIG. 1.—Extensive alopecia and crusted lesions along the dorsal aspect of the back.

Pathology.—At necropsy the skin was diffusely but irregularly and asymmetrically affected by patches of partial-to-complete alopecia. The lesions were most apparent over the dorsal and lateral aspects of the head, neck and trunk (Fig. 1). There was moderate, diffuse erythema adjacent to oozing, raised discoid ulcers, the largest of which measured 2·0 cm in diameter. Some of these raised areas were covered by dry crusts which could be removed to reveal underlying dull pink, apparently healing epidermis. There were similar oozing and healing areas at the mucocutaneous junctions of the eyes, mouth, anus and vulva. The ulcerated lesions had a halo of subcutaneous erythema of a few mm in width. Throughout the body, lymph nodes were of normal size; normal corticomedullary demarcation was apparent on their cut surfaces. Unrelated necropsy findings included: splenic nodular hyperplasia and capsular siderofibrosis; bilateral chronic osteoarthrosis of the hip joints; cardiac valvular endocardiosis; gingival fibromatous epulides; purulent infection of the right anal sac; and adrenocortical nodular hyperplasia.

Histologically, the florid cutaneous lesions had extensive necrosis, ulceration (Fig. 2) and desquamation of the epidermis. In the underlying dermis there was variable slight oedema and diffuse accumulation of mononuclear cells (Fig. 3). The diameter of these

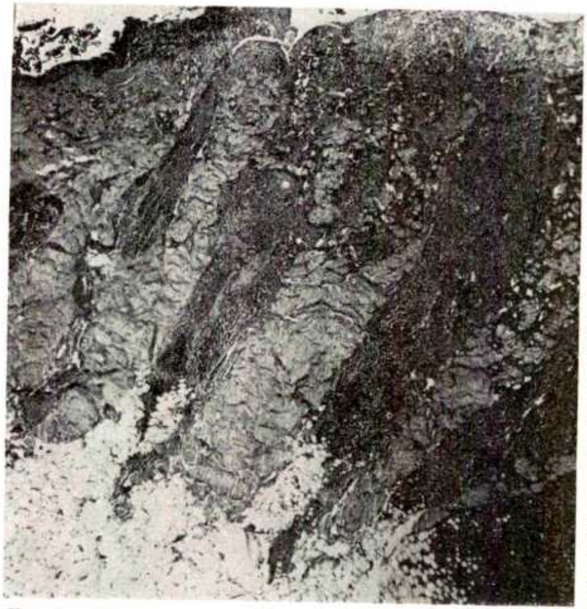

FIG. 2.—There is extensive necrosis and ulceration of the epidermis; beneath the ulcer there is diffuse cellular infiltration of the dermis by histiocytic cells. (H. and E. × 40.)

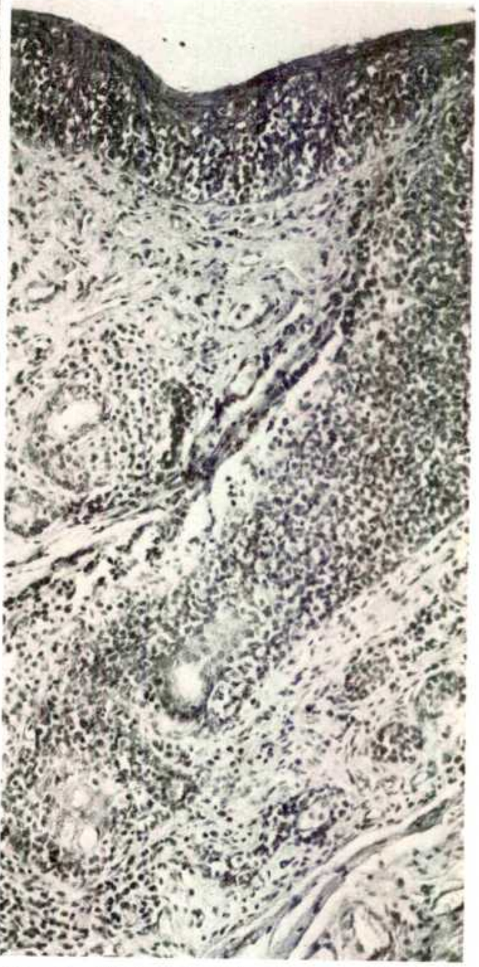

FIG. 4.—Histiocytic cells are present in both epidermis and adnexae. There is a light infiltration of the dermis. (H. and E. × 155.)

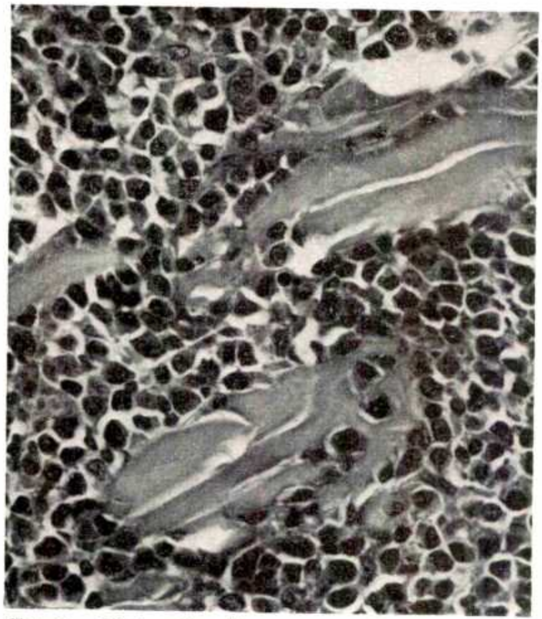

FIG. 3.—Histiocytic cells are present between bands of dermal collagen. (H. and E. × 450.)

cells was approximately 10 μm. Their nuclei varied from round to irregular or reniform, with coarse granular chromatin and large nucleoli. Mitoses were plentiful. The cytoplasm was abundant, eosinophilic and granular. These cells were interpreted as being histiocytic and are referred to as such subsequently. Similar cells were in the basal layers of the epidermis, within adnexal structures (Fig. 4), and extended for a variable distance into adjacent loose connective tissue and between muscle bundles. Acanthosis and clubbing of the epidermis were conspicuous in some areas (Fig. 5); in others there was epidermal atrophy. Focal intra-epidermal collections of histiocytic cells, resembling Darier-Pautrier micro-abscesses, were common (Fig. 8). The dry, crusted lesions also had histiocytic cells in the epidermal structures (Fig. 6, 7), but there was relatively less infiltration of the dermis. Lymphocytes and plasma cells were conspicuous in the subcutaneous adipose tissue in some sections. In the mucocutaneous junction of the anus there was particularly heavy accumulation of histiocytic cells; these involved the epidermis, as described elsewhere, but not the rectal mucosa (Fig. 9). They extended widely between adjacent para-anal gland tissue.

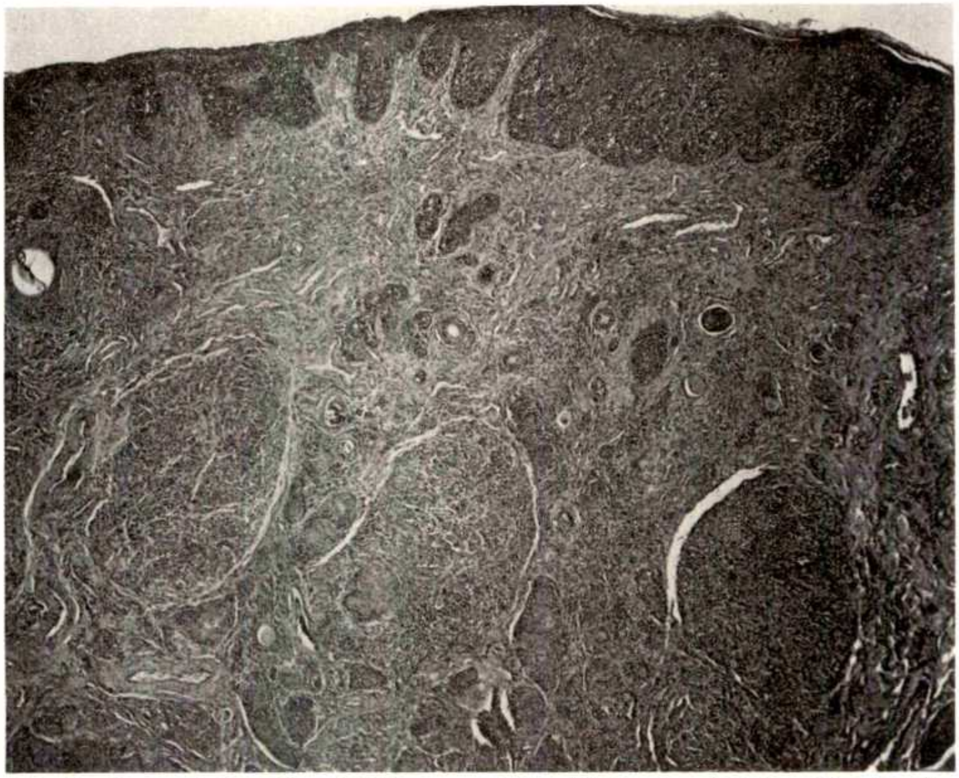
FIG. 5.—Para-anal skin. There is diffuse accumulation of histiocytic cells in the epidermis. At the right there is marked acanthosis. (H. and E. × 45.)

Extensive histological examination was made of lymph nodes and bone marrow. Lymph nodes were architecturally normal, with minimal sinus catarrh, erythrophagocytosis, mild haemosiderosis, and accumulation of plasma cells within medullary cords. Bone marrow, spleen and liver were normal.

DISCUSSION

At a descriptive morphological level the cutaneous lesions consisted of a diffuse infiltration with histiocytic cells. It is presumed that the epidermal changes, leading to necrosis and desquamation, were secondary to the dermal involvement, possibly as a sequel to interference with epidermal nutrition. The evidence of epidermal healing, together with the regression of the underlying histiocytic infiltration, suggested that the lesion might have resolved, at least temporarily, if the dog had been permitted to live. We have only negative evidence concerning the possibility of an infectious cause; the cutaneous lesions do not resemble mycotic or bacterial infection, there was no response to broad-spectrum antibiotics and corticosteroids, and no pathogenic micro-organism was recovered in culture.

In establishing a morphological differential diagnosis lymphosarcoma, mastocytoma, and an atypical form of canine cutaneous histiocytoma were considered. Complete post-mortem and histological examination of lymph nodes, spleen and bone marrow allowed the former 2 conditions to be excluded. The evidence regarding the absence of lymphosarcoma is emphasized because the term " mycosis fungoides " has been used, in the veterinary literature, to describe cases of canine lymphosarcoma with cutaneous involvement (Muller and Kirk, 1969; Wirth, 1920). Cutaneous lymphosarcoma in the dog is relatively uncommon. In 93 cases of lymphosarcoma recorded in California only 10 had involvement of the skin (Dorn *et al.*, 1967), and the data concerning concomitant lymphoma in other organs of these 10 cases is not available (Taylor, personal communication, 1970). In the material submitted to the University of Pennsylvania between 1963 and 1969 there were only 2 adequately documented cases of cutaneous lymphosarcoma in a total of 166 cases of canine lymphosarcoma: both of these dogs had overt lymphosarcoma involving lymph nodes (unpublished data—

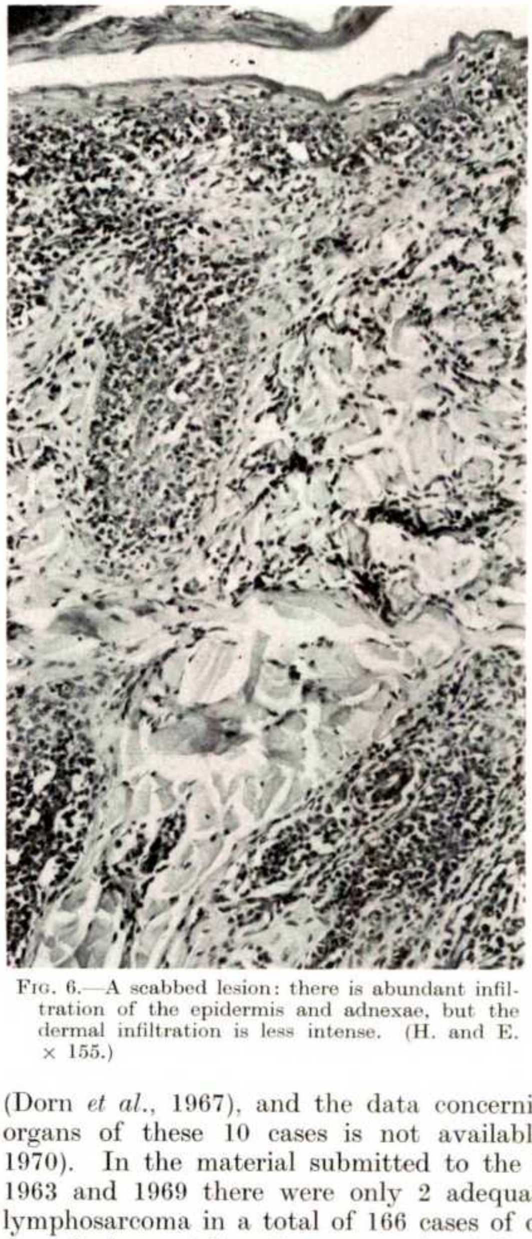

FIG. 6.—A scabbed lesion: there is abundant infiltration of the epidermis and adnexae, but the dermal infiltration is less intense. (H. and E. × 155.)

Section of Epidemiology, University of Pennsylvania, School of Veterinary Medicine). In another series in Glasgow there were 3 cases of cutaneous involvement in 61 dogs with multicentric canine lymphosarcoma (Crighton, 1965).

An acceptable case of primary cutaneous lymphosarcoma in the dog has recently been published (Howard *et al.*, 1969): a Boxer bitch aged 9 years had multiple firm cutaneous nodules consisting predominantly of large lymphoblasts and lymphocytes; epidermal invasion by the tumour cells was not a feature. Although lymphoblasts were seen in pulmonary blood vessels and in hepatic sinusoids, frank lymphosarcoma of lymph nodes was not observed. On both gross and microscopic grounds the case described by Howard *et al.*, seems to be distinguishable from the case described in this report.

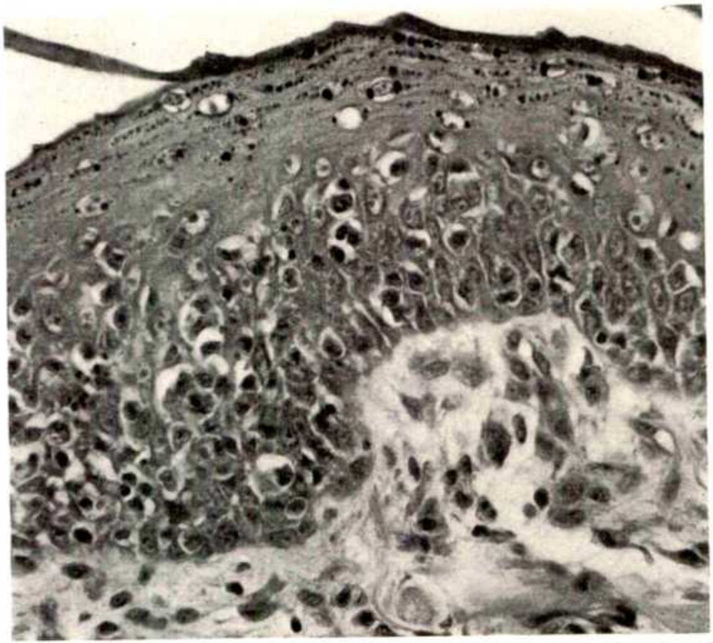

FIG. 7.—Heavy histiocytic cellular infiltration of the thickened epidermis. (H. and E. × 375.)

The presence of overt lymphosarcoma (as judged by histological criteria) was excluded in this case, but it is possible that the condition we describe is a premalignant form of lymphosarcoma. Unfortunately the animal was not allowed to survive, so this possibility must remain in doubt. Although the lesion appears to be a predominantly histiocytic infiltration of the dermis, it is quite unlike canine cutaneous histiocytoma, which usually presents as a solitary, elevated, rapidly-growing nodule, which may spontaneously regress. Epidermal infiltration by the cells of canine histiocytoma can occur, but it is not usually a striking feature of the lesion (Kelly, 1970).

We have concluded that this cutaneous lesion cannot convincingly be assigned to any existing nosological entity for the dog but that it has some similarities to mycosis fungoides in man: the protracted clinical course with temporary partial regression; diffuse dermal infiltration by histiocytic cells and extension of the cells into the epidermis; absence of concomitant reticuloses. The histiocytic cells were less polymorphic than those often seen in human mycosis fungoides; this

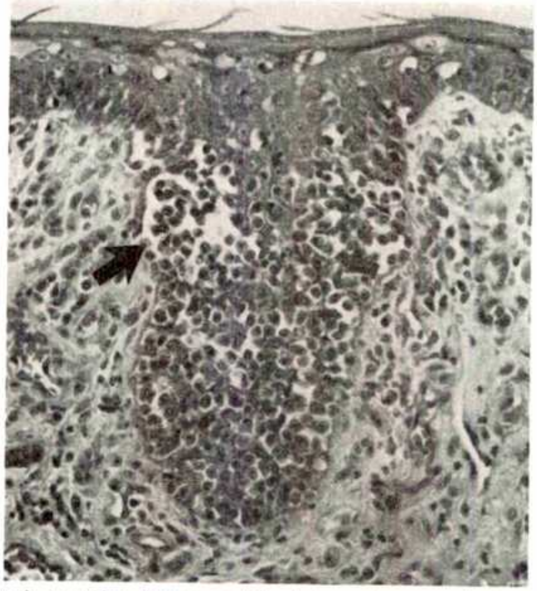

FIG. 8.—The epidermis contains diffuse and focal (arrow) accumulations of histiocytic cells. (H. and E. × 310.)

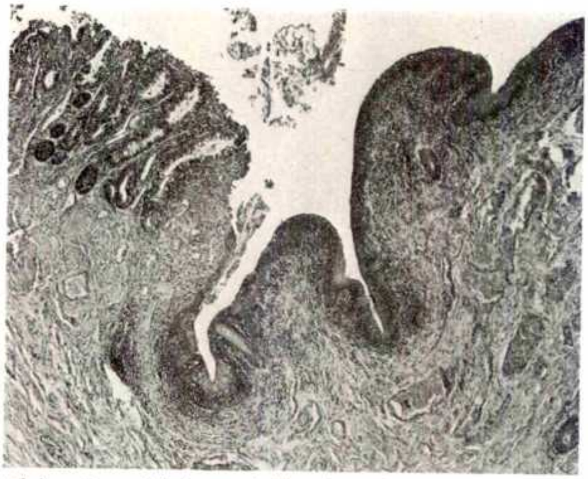

FIG. 9.—Ano-rectal junction with heavy infiltration of epidermis and dermis. (H. and E. × 45.)

polymorphism is known to be a variable feature in man. It is recognized that comparisons should not be based on morphological similarities alone, but we consider this case to be sufficiently unusual to warrant being reported. It is hoped that attention may be drawn to similar cases which might serve as a useful model for more fundamental studies on the nature of the tissue changes in human mycosis fungoides and on the nature of any possible relationship to other reticuloses.

We are grateful to the following pathologists who reviewed the sections from this case: Professor Eugene J. Van Scott, Skin and Cancer Hospital, Temple University, Philadelphia; Dr I. W. Whimster, Department of Pathology, St Thomas's Hospital, London; Dr Donald Alcott, Santa Clara Valley Medical Centre, San Jose, California; Dr Dee O. N. Taylor, Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, California; Dr E. Wilson Jones, St John's Hospital for Diseases of the Skin, London.

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Pharmacology and Treatment

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GRENZ RAY TREATMENT OF RECURRENT HERPES SIMPLEX

A. G. KNIGHT

Summary.—Twenty-five patients with recurrent herpes simplex were treated with Grenz rays and assessed at intervals of up to 2 years. Fifty % were cleared, and a further 30% improved. A control group of 10 patients, on no treatment whatsoever, remained unchanged over a period of 2 years.

GRENZ rays have been used for many years in the treatment of herpes simplex infections of the skin. However, there are no reports in the literature on the results of such therapy. There are reports on the use of x-rays. Robert (1940) used 90 kV, target distance 23 cm, and either a single dose of 250 R or 2 doses of 155 R. Of 19 patients observed, for an average of 2 years, 8 cleared, 8 were improved and 3 failed to improve. Frank (1949) used 80 kV at a target distance of 20 cm, and either 1 or 2 doses of 130 R. Of 12 patients observed, over an average of 2 years, 10 cleared and 2 improved. Finally, Hall (1938) assessed the speed of recovery of individual herpetic lesions, using 130 kV, target distance 30 cm, in a single dose of 375 R.

TABLE I.—*Comparison of Depth Dose of Superficial x-rays (Field 3 cm²)*

	Grenz		Contact	Conventional
Kilovoltage	10	15	50	100
Target surface in cm (distance)	10	10	2	20
% surface	100	100	100	100
% 0·05 cm (0·5 mm)	28	53	85	94
% 0·3 cm (3·0 mm)	1	8	46	73
% 0·5 cm (5·00 mm)	0	2	32	62
% 2·0 cm (20·00 mm)	0	0	5	28
% 5·0 cm (50·00 mm)	0	0	0	9

The causative virus of herpes simplex is present in the epidermal vesicles of herpes simplex (Sanders *et al.*, 1953), and this is the level at which maximal effect is needed. Grenz rays are very soft x-rays, low in penetrating power, and absorbed superficially. They are generated by tensions of 8–17 kV applied to tubes whose windows will permit the emergence of such soft rays in clinically usable amounts (Bucky, 1929). They exert their most intense effect in the first 0·5 mm of tissue. Jennings (1953) compiled all the known data and came to the conclusion that only a very small proportion of rays penetrate to 3 mm, which is the level of the deep vascular plexus. Southwood (1955) measured the thickness of facial skin, and found the average thickness of epidermis was 0·05 mm, and the dermis 1·4–2·2 mm.

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Grenz rays are preferable to x-rays in their absence of epilation and deep radiation sequelae (Goldsmith, 1943; MacKee and Cipollaro, 1935). High doses used for carcinomata can, however, cause superficial atrophy, telangiectasia and pigmentation. Two cases of squamous cell carcinoma, following chronic exposure to Grenz rays, have been reported. Both patients were dermatologists, one of whom was estimated to have received a total of 60,000–70,000 R in 4 years (Kalz, 1959; Sagher, 1962) (Table I).

METHODS

Thirty-five patients were examined, 12 male and 23 female, with an age range of 11–43 years, the average being 23 years. All had herpes simplex, which had recurred more than 3 times per year for at least 3 years. Twenty-five patients received Grenz ray therapy, and 10 were left untreated, acting as controls. The 2 groups were not strictly comparable, as the untreated group consisted of patients with lesions near the eye, or lesions not sufficiently troublesome to necessitate therapy, or patients with a dislike of radiation therapy. The average frequency of attacks amongst those treated was 10 per year, and between 4 and 5 among those left untreated. The average duration of the illness in the treated group was 10 years; in the untreated group it was 15 years.

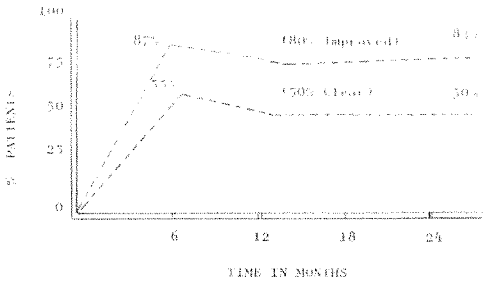

FIG. 1.

Grenz rays of 10 kV, target distance 10 cm, were applied to the area affected in 4 doses of 200 R at fortnightly intervals. Patients were assessed at 6 months, 1 year and 2 years. They were classed as improved if they had longer intervals between attacks, if the lesions were smaller and of shorter duration (Fig. 1).

RESULTS

The 10 control patients were unchanged over a period of 2 years. Of the 25 treated patients, 55% were clear at 6 months, and a further 32% were improved. Twenty patients were seen at 1 year, and 50% were clear, while a further 30% were improved. Only 12 of the patients studied had been followed for 2 years; but, of these, 6 were clear, and only 2 failed to show any improvement.

TABLE II.—*Results*

	Treated group	Untreated controls
Number	25 patients	10 patients
Sex	9 male, 16 female	3 male, 7 female
Age range	11–43 years	13–45 years
Average age	23 years	23 years
Duration of illness (average)	10 years	15 years
Frequency per year	10	4–5
Response over 2 years	50% clear 80% improved	Unchanged

Of the total 25 patients who received Grenz ray therapy, 6 had 2 courses because of failure of the initial therapy. Two patients had occasional herpetic lesions outside the treated areas, and 4 patients had a temporary exacerbation of the lesion during treatment with Grenz rays (Table II). Of the 6 patients who received a second course, 8 to 18 months after the first, 2 were cleared and 4 improved.

DISCUSSION

There are many treatments for herpes simplex. Anderson *et al.* (1950) used a vaccine, made of killed virus, on 16 children under the age of 1 year, which had no effect in preventing herpetic infections. Vaccination of 2 adults, suffering from genital herpes, with unmodified vesicle fluid, led to active herpes at the site of the injections, and no improvement in the other lesions (Lazar, 1956).

Kaufman (1963) reported on idoxuridine, a chemotherapeutic agent which was effective against herpetic keratitis in rabbits. The drug acts by blocking the synthesis of thymidine necessary for the formation of DNA of the virus. Idoxuridine has been used in herpetic keratitis with good results (Kaufman *et al.*, 1962). However, results on the skin were not so encouraging. Juel-Jensen and MacCallum (1964) found no difference between the active and placebo creams. Idoxuridine is remarkably soluble in DMSO (dimethyl sulphoxide). MacCallum and Juel-Jensen (1966) used a 5% solution in 100% DMSO, in a double-blind trial. When applied thrice-daily to a recent herpetic infection, it increased the rate of healing by 63%, as compared with DMSO alone.

None of the above forms of therapy appears to prevent the recurrent nature of herpes simplex infections. From the evidence presented above, Grenz rays have a place in preventing recurrences, as 50% of patients, over a 2-year follow-up, are clear of the infections. Critics may say that a better assessment would have been possible if both groups had actually been confronted with the Grenz ray apparatus. However, the conclusion drawn from this trial is that Grenz rays are a safe and profitable form of treatment for recurrent herpes simplex.

I would like to thank Dr C. F. H. Vickers for his helpful advice, and the Wellcome Trust for their support generally.

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Case Reports

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MICROSPORUM EQUINUM INFECTION OF THE SCALP IN AN ADULT

K. J. O'GRADY, M. P. ENGLISH AND R. P. WARIN

Summary.—Widespread infection of the scalp and face with *Microsporum equinum* is described in a woman aged 55. She was on regular therapy with prednisone, having undergone hypophysectomy for breast carcinoma with secondary deposits, 4 years previously.

CASE REPORT

A WOMAN aged 55 had a left mastectomy for carcinoma in 1962. This was followed by cobalt treatment to a mass on the left side of the neck in August 1965. When multiple secondaries developed in 1966, hypophysectomy was performed, and she was subsequently given replacement therapy with cortisone, 75 mg daily. From 1968 she had received prednisone, 7·5 mg daily and she also required thyroxine and syntopressin.

When the patient first attended the Dermatology Department in April 1971 with an extensive erythematous, irritating rash involving the scalp and upper forehead, she was remarkably fit generally, with no clinical evidence of secondaries. The rash had begun in mid-December 1970 as itchy, red papules on the cheek and adjacent upper lip; the rash then spread to her forehead and neck. The eruption on the face gradually cleared, but the scalp became involved in February. No lesions developed elsewhere.

The patient had her own horse, which she groomed and rode regularly. In November, about one month before her own eruption appeared, this horse had had a patch of scaling and loss of hair, 2·5 cm in diameter on the girth region. It responded to treatment with iodine. Unfortunately, by the time the patient attended hospital, the horse had been sold.

On examination, the patient's scalp showed diffuse scaling and some slight patchy hair loss, and a scaly, slightly erythematous eruption on the upper part of the forehead. Fluorescence of a few short scattered hairs under Wood's light was noted, and hairs and scrapings were examined mycologically. Microscopy showed a small spore ectothrix infection of the hairs. In culture almost every inoculum, both of scrapings and from brushed samples, yielded *M. equinum*. The fungus closely fitted the definitive description given by Stockdale (1965). On glucose-peptone agar, macro-conidia were very scanty but larger numbers were found when the fungus was grown on cornmeal agar.

The patient was treated with Grisovin F.P. 500 mg daily, and after 4 weeks no fluorescence was demonstrable and no fungus was found on microscopy or culture of scrapings or brushings.

DISCUSSION

Ringworm acquired from horses is seen only occasionally in dermatological clinics, the species usually involved being *Trichophyton equinum*. No infections

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with *M. equinum* have been seen in the mycological diagnostic service centred in Bristol and covering a large part of the West Country in the 18 years of its existence, although the fungus has been isolated from horses in the area on a number of occasions. A search of the literature has disclosed only 3 records of human infections by this fungus, all in children. Medvedeva (1964) notes 2 scalp infections in the USSR, and Austwick and Pepin (1967) found one infection of the glabrous skin in Britain. However, outbreaks of *M. equinum* infection of horses in this country are not uncommon. The *Report on Animal Health* (1969) records 11 cases in 1967. The apparently low infection rate in man may reflect a genuine resistance to this fungus, but it is also possible that the lesions produced are normally very mild and not therefore seen in dermatological clinics. Also, many workers consider *M. equinum* to be non-specific with *M. canis*. It is, therefore, likely that a number of cases reported in the literature of *M. canis* ringworm contracted from horses were, in fact, due to *M. equinum*. This is a further reason for supposing that the condition may not be as rare as it would appear to be.

It is very rare for other *Microsporum* spp. to affect the adult scalp, although the infection may be common in children. If the normal adult has a similar resistance to *M. equinum* it seems likely that the underlying endocrinological state in our patient has played an important part in allowing the development of this very unusual, widespread scalp infection.

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JADASSOHN TUMOUR ARISING AT SMALLPOX VACCINATION SITE

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Summary.—A tumour arising at the site of a smallpox vaccination performed 43 years previously was found on histological examination to be a seborrhoeic keratosis with features of the intra-epidermal " epithelioma " of Jadassohn.

THE development of skin tumours at the site of a former smallpox vaccination is now well documented (Marmelzat, 1968; Reed and Wilson-Jones, 1968). More than half of the reported cases have been basal cell carcinomata, with malignant melanomata and squamous cell carcinomatas accounting for most of the remainder.

We report here a patient who, 43 years after smallpox vaccination, developed at the vaccination site a seborrhoeic keratosis showing characteristics of the so-called " intra-epidermal carcinoma " of Jadassohn.

CASE REPORT

A man aged 74 was vaccinated in the first world war. Ten years ago the surface of the residual scar started to become roughened. During the past year this roughness and scaling gradually extended outwards from the vaccination site.

On examination, the lesion, which measured 3·5 cm in diameter, was raised, erythematous and scaly and had a well defined edge (Fig. 1). There were no similar lesions elsewhere on the skin. Bowen's disease and seborrhoeic keratosis were considered as the most probable diagnoses.

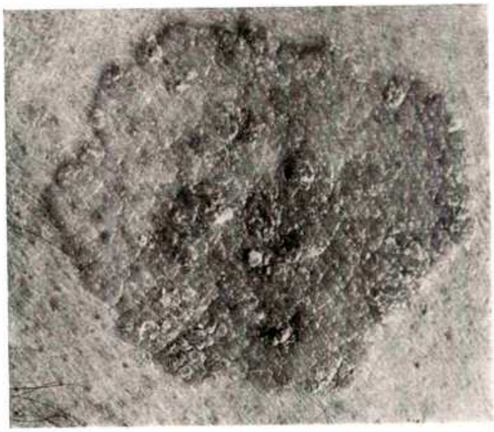

FIG. 1.

The lesion was excised with a 1 cm marginal clearance and a skin graft applied. Histological studies were made from 6 different areas of the lesion. These showed a central area of scar tissue about 0·5 cm in diameter over the centre of which the epithelium was a little flattened (Fig. 2). At the margins of the scar the epithelium showed a diffuse hyperplasia, which spread to the margin of the lesion and had the appearance of a seborrhoeic keratosis with numerous well-defined nests of basaloid cells surrounded by squamous cells, the picture of intra-epidermal basal cell epithelioma of Jadassohn (Fig. 3).

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DISCUSSION

The reason for the development of neoplasms at vaccination sites remains unknown. Clearly epidermal changes are in part influenced by the underlying dermis, in this case scar tissue. In discussing this phenomenon Reed and Wilson-Jones (1968) have already pointed out the association between histiocytomata and development of overlying basal proliferation mimicking basal cell carcinoma. It is known that changes resembling seborrhoeic keratoses can also occur in this situation (Schoenfeld, 1964). A similar mechanism may explain neoplasia at vaccination sites. Reed and Wilson-Jones (1968) suggest that, in basal and squamous cell carcinomata, sunlight exposure is an important aetiological factor. Their patients, all residents of California, showed other evidence of damage from sunlight. Our patient, however, had never been resident outside the United Kingdom.

Marmelzat (1968), although mentioning solar exposure, preferred to speculate on the possible role of vaccinia virus in initiating neoplasia, and a virus-induced mutation of melanocytes following the vaccination reaction has been postulated in a case of malignant melanoma (Reed and Wilson-Jones, 1968).

The Jadassohn tumour was formerly considered to be an intra-epidermal basal cell epithelioma (Jadassohn, 1926). Mehregan and Pinkus (1964) have demonstrated that lesions designated as intra-epidermal carcinomata fall on further scrutiny into a number of different categories, the majority being seborrhoeic keratoses.

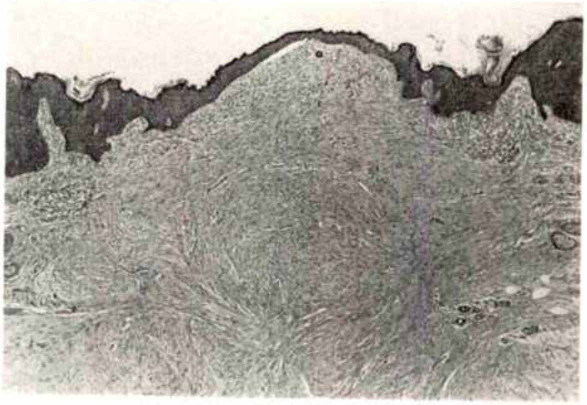

Fig. 2.

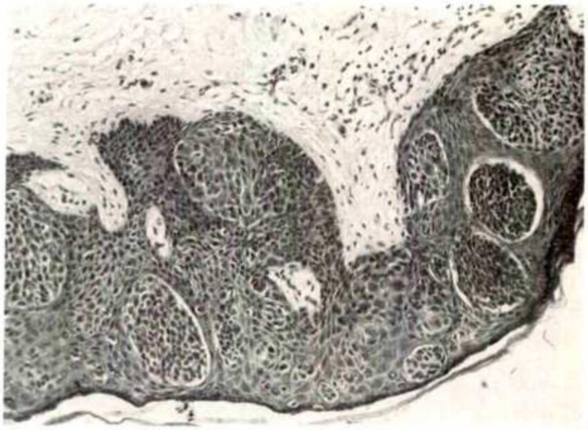

Fig. 3.

Whereas previously reported tumours at vaccination sites have been malignant, the present case is of interest in that it is an uncommon histological variant of a benign tumour.

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A HISTORY OF DERMATOLOGY IN BRISTOL AND THE WEST OF ENGLAND

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THE area of the West of England included in this article comprises the counties of Cornwall, Devon, Somerset and Gloucester, and Bristol—which is a county in itself. The estimated population of this area in 1967 was 3,057,000, whereas in 1841 it was 1,741,104, and this rate of increase of about 1,317,000 in 116 years is considerably less than in many parts of the country. Bristol, with a total of 429,000 persons, is the largest city, with Plymouth—249,000—second. The cities of Bath, Gloucester and Exeter have populations of 85,870, 89,620 and 92,550 respectively. The number of people in the area is increased enormously in the holiday period, especially in Devon and Cornwall, when all the seaside resorts are crowded; this causes much seasonal extra work for the local hospitals.

The two old established general hospitals in Bristol are the Royal Infirmary and the General Hospital. They were merged in 1939 to form the Bristol Royal Hospital, which is part of the United Bristol Hospitals.

THE BRISTOL ROYAL INFIRMARY

The Bristol Royal Infirmary is one of the oldest provincial voluntary hospitals, and was founded in 1736 (Smith, 1917a). Out-patients were first accepted in June, 1737, and In-patients in December of the same year. From the earliest days pupils were attached to the apothecaries and surgeons; physicians do not appear to have taught pupils until about 1829. Lectures were given by members of the staff from 1746 onwards, and Thomas Shute (Smith, 1917b) used to get his " subjects " by wagon from London; the Anatomy Act was not passed until 1832! These lectures were carried on by his successor, who called his establishment " The School of Anatomy and Medicine ", which was recognized by the Apothecary's Hall in London. A rival establishment was the Bristol Medical and Surgical School, which was recognized by the Royal College of Surgeons. In 1833 the 2 institutions combined as the Bristol Medical School, which was affiliated to the University College founded in 1876 and which became the Faculty of Medicine in 1893, and of the University of Bristol founded in 1909. Women students were first admitted in 1906.

Dr Edward Lyne, Physician 1758–1772, earned a high reputation as a specialist in dropsy (Smith, 1917c). The secret of his treatment was " Bristol Milk ". He himself never saw patients after 2.00 p.m., when he had his dinner, accompanied by a bottle of his " cure ", which he described as a generous wine from Lisbon. It is interesting that the Infirmary patients were allowed 4 glasses of beer per day, and presumably the " cure " was for his private patients.

A new building was started in 1784, with additional blocks in 1788 and 1806. Further additions were made in 1859, and in 1912 the new King Edward VII Memorial building of 300 beds was opened as a Military Hospital—this is still known as the " new building ".

The Honorary Staff were appointed for a maximum of 20 years until 1877, and subsequently there was an age limit for retirement, which persists today. All appointments as Consultants were honorary until the introduction of the National Health Service.

In the early days, as soon as a man qualified he was allowed to wear his top-hat in the wards!

A Skin Department was formed at the Infirmary in 1880, under the care of Dr Henry Waldo—a physician with an interest in diseases of the skin.

THE BRISTOL GENERAL HOSPITAL

Early in the nineteenth century a second hospital was required to deal with the volume of work. The Bristol General Hospital was opened in 1832 with 20 beds. The property cost £3,750, and part of it—a timber yard and a warehouse—was at once sublet. Alterations cost £485, and a well was dug for £35 (Saunders, 1965).

In 1858 a new building was erected on a site adjacent to the old hospital; this forms the core of the present hospital, with additions added. In 1873 a new Outpatient Department was added, and further wings in 1888 and 1914, which brought the total beds to 180.

In 1902 a " chamber was set apart for electrical and hydropathic treatment. Here are installed some of the most modern Finsen light lamps " (Local Medical Notes—1902). Dr Kenneth Wills, the dermatologist, was an assistant in this department, where he treated lupus vulgaris with both x-rays and u.v. light.

The General Hospital was, from the first, supported by Nonconformists and Liberals, while the Infirmary was supported by the Church and Tories. It is said that, when the Infirmary was granted the prefix Royal in 1850, a hospital supporter remarked: " The patients who want a sovereign remedy will go to the Royal Infirmary, but those who want a radical cure will go to the General Hospital ".

A Skin Department was formed in 1884 (Symes, 1932) under the care of Dr Alfred James Harrison, a physician with an interest in diseases of the skin, who later devoted himself entirely to dermatology.

Since the merger of the above 2 hospitals in 1939, the Dermatological Outpatient Department has been in the General Hospital branch, and the beds at the Royal Infirmary.

Other hospitals in Bristol are visited by the dermatologists, including Southmead, which now takes students, Frenchay, specializing particularly in plastic surgery, accident and neurosurgery, and Manor Park, a geriatric hospital.

THE BRISTOL DISPENSARIES FOR DISEASES OF THE SKIN

There were two dispensaries in Bristol for the treatment of skin diseases. The first was opened in 1883 by Dr John Broom, M.D., Brux. (1842–1900) as the Bristol Institution for Diseases of the Skin, 21 Nicholas Street, moving to 6 Colston Street in 1888 as the Bristol Institution for Skin and General Diseases until 1893, when it was moved to 13a College Green. Dr Broom practised in

Sheffield before moving to Bristol in 1877. He contributed articles to the *Lancet* and *British Medical Journal*, but not on dermatological subjects. Seven of his suggestions for improving the Medical Act of 1883 were incorporated in the Medical Act 1886 (1883). The Dispensary closed in 1900.

The Dispensary for the Treatment of Diseases of the Skin opened in 1885, under the care of John Hancocke Wathen (1845–1906) in Park Row, and closed in 1895. Dr Wathen was an original member of the Dermatological Society of Great Britain and Ireland in 1894, and in 1898 presented his own experience of a local contact dermatitis due to iodoform gauze (1898).

FIG. 1.—William Budd 1811–1880.

PHYSICIANS AND DERMATOLOGISTS

Bristol

Thomas Baynton, a medical practitioner, who had been an apprentice at the Infirmary, wrote a book in 1797 entitled "A New Method of Treating Old Ulcers of the Legs". His method was to pull the edges of the ulcers together with diachylon plaster. This was combined with a recognized treatment of firm calico bandaging from the toes to the knees. He also advocated hourly moistening of the bandage with lead lotion or, better still, plain water, to reduce the inflammation. He considered that walking helped, and that support, after cure, was necessary.

William Budd, F.R.S. (1811–1880) was appointed Physician to the Infirmary in 1847, and achieved fame at home and abroad for his work on infectious fevers and in epidemiology. As a student in Paris, he worked at the Hôpital St Louis where he received dermatological instruction from Biett. He also attended several London hospitals before going to Edinburgh, where he was awarded his M.D. with a gold medal for his thesis. His first paper on infectious diseases was on malignant cholera (1849), when he maintained that it was due to a living organism, specific for this particular disease, which at that time he thought was a fungus. He showed under the $\frac{1}{8}$-in objective of the microscope "peculiar objects" in the "rice water" stools, which he said caused the disease. The objects were also seen in specimens of drinking water where the disease was rife. He recommended disinfection of excreta, and the provision of a pure water supply to prevent the spread of infection. In 1856 he reported the same method of spread of typhoid fever.

He addressed the annual meeting of the British Medical Association in 1862 on anthrax, mentioning 23 cases, of which 22 were fatal (1863). He noted that cases were contagious from animal to man, man to man, and man to animal, and also that the hair of infected animals carried infection. He considered that the disease was carried by minute living organisms. Two other notable publications were on scarlet fever (1871a) and an outbreak of cholera in Bristol (1871b).

His only dermatological writing apart from his work on anthrax was never published, but is an undated manuscript of an outbreak of scabies in Bristol and the surrounding district (Goodall, 1936). He found that the condition affected rich and poor alike, and occurred in epidemics.

At a meeting of the Infirmary Medical Committee in 1858 he suggested that all prescriptions in the Infirmary Pharmacopoeia should be written in English; this met with little support.

He was elected a Fellow of the Royal Society in 1871 for his work on infection and epidemiology.

Alfred James Harrison (1836–1924) studied at Birmingham Hospital and Guy's Hospital. He took the Conjoint Diploma in 1858 and the London M.B. 3 years later. He was appointed Assistant Physician to the General Hospital in 1879, with charge of patients suffering from skin diseases. In 1901 he was appointed as the first dermatologist to the hospital, and retired 3 years later. His first dermatological publication was on the treatment of tinea tonsurans, with solutions forming biniodide of mercury (Harrison, 1885). Like many of his medical colleagues, he took a great interest in the welfare of the animals in the Bristol Zoo; this still pertains to this day. His Presidential Address to the Bristol Medico-Chirurgical Society in 1894 was "Comparative Pathology" (Harrison, 1894a). He records his post mortem findings in Hannibal, a male lion, in whom the spleen weighed 21·5 lb instead of the normal 1 lb. Another lion had a suppurative pericarditis, and many of the monkeys died of tuberculosis. He gave anaesthetics of chloroform to the animals, including an Arabian gazelle, for a Caesarean section, and to a cockatoo!

FIG. 2.—Alfred James Harrison 1836–1924.

In 1894 the British Medical Association met in Bristol, and in his Presidential Address to the Dermatological Section he stated: "There is no case of eczema that does not admit of cure"—a statement with which most of us would not agree (Harrison, 1894b). His further remark is still appropriate today: "Remedies ever new are being advertised, most of them have their little day, and then fade away and cease to be". He gave the Long Fox Memorial Lecture in 1906, in which he considered that dermatitis from caterpillars was due to formic acid in the hairs (Harrison, 1906). He was President of the Dermatological Society of Great Britain and Ireland in 1901.

Henry Waldo (1837–1935) was educated at Bristol Grammar School, Bristol Medical School, St Bartholomew's Hospital and Aberdeen, qualifying at Aberdeen in 1871; he gained his M.D. 2 years later, and the M.R.C.P. in 1888. He was appointed Assistant Physician to the Royal Infirmary in 1873, and Physician 3 years later. In 1880 he was given charge of patients suffering from diseases of the skin. He was the first physician to be appointed without a 20-year limit.

FIG. 3.—Henry Waldo 1837–1935.

He wrote frequently for the Journals—13 publications being on dermatological subjects, and 22 on general medical problems.

In 1901 he addressed the Annual Meeting of the Dermatological Society of Great Britain and Ireland on lupus erythematosus (Waldo, 1901). He maintained that, although many physicians, including the President, Dr A. J. Harrison, considered that lupus vulgaris and lupus erythematosus were of tuberculous origin, he was sure that they were unrelated, and he pointed out that the histology was quite different. In 1906 he was himself president of the Society. The next year he was President of the Bristol Medico-Chirurgical Society, and gave his address on syphilis (Waldo, 1907). He mentioned the discovery, 2 years previously, by Fritz Schaudinn of the *Spirochaeta pallida*, which had greatly increased the knowledge of the disease known as " the great imitator ".

Theodore Fisher (1863–1949) qualified with honours at Guy's Hospital in 1887, and was awarded the gold medal in the same year. He was elected an original member of the Dermatological Society of Great Britain and Ireland in 1894, whilst working as Clinical Assistant at the Hospital for Diseases of the Skin, Blackfriars. He described 4 cases of erythema of the face of butterfly distribution, which he said was not like erysipelas or lupus erythematosus, but more like very acute rosacea (1893). All were very ill, with stomach or intestinal disease, and developed the rash a day or so before death, which he felt was due to failure of the vasomotor system.

FIG. 4.—Walter Kenneth Wills 1873–1968.

He was appointed Pathologist to the Royal Infirmary in 1895, and was also physician to Out-patients at the Hospital for Sick Children and Women. In 1905 he moved to Sidcup, and was elected to the Staff of the East London Hospital for Children. He was elected F.R.C.P. in 1908.

Walter Kenneth Wills (1873–1968) succeeded Dr Harrison in the Department at the Bristol General Hospital in 1904, and was also Skiagraphist and Assistant Physician to the Electrical Department in 1903 and 1904. He trained at Cambridge, Guy's Hospital and Vienna, taking the Conjoint Diploma in 1898 and M.B., B.Chir. in 1899. He took a great interest in x-ray treatment, and in 1903 reported cases of lupus vulgaris treated by this means, stating that he felt that x-rays had a peculiar selective affinity for lupus tissue (Wills, 1903). This treatment was often combined with u.v. light therapy, and it is difficult to assess from his early articles, what each type of therapy achieved. At this time the u.v. lamps had either iron or carbon electrodes, and he devised a water-cooled lamp with carbon electrodes, with soft iron knitting needles running through the middle of the carbons. He mentions that his experience with the u.v. lamp in lupus erythematosus was not encouraging! In 1904 he found that the treatment of rodent ulcers with x-rays was difficult, because of the uncertainty of accurate dosage (Wills, 1904). Most of us, working 30 to 40 years later with Pastille doses, found the dosage somewhat erratic even then, as it depended solely on colour change of the " Pastille ". Also in 1904 he had been using x-rays for depilation in sycosis barbae and tinea tonsurans (Wills, 1904). The recurrence rate of rodent ulcers treated by radiotherapy was 20–40%. In the treatment of 80 cases of lupus vulgaris, treated between 1901 and 1905, he obtained better results with x-rays than with u.v. light (Wills, 1907). In 1909 he investigated an outbreak of " barley itch " in dockers unloading barley from north-west Africa (Wills, 1909). This was a very irritating urticarial type of rash on the exposed parts, starting within half an hour of handling barley. The grain was examined under a lens and microscopically, and found to have sharp vegetable hairs, but no mites were found. His investigations showed that cowhage, which grows in tropical Africa, will produce the same type of eruption and, microscopically, revealed similar hairs.

Dr Wills was associated with the Severn Division of the R.N.V.R. from its inception, and was mobilized at the outbreak of the First World War. He was awarded the O.B.E. for his distinguished war service.

He was president of the Bristol Medico-Chirurgical Society in 1928, and his Presidential Address was on " Developments in Dermatology in the Past Thirty Years " (Wills, 1928). He reminded his audience of the discovery of *Spirochaeta pallida* in 1905, the Wassermann reaction 2 years later, and, in the same year, the introduction of the treatment of syphilis by Salvarsan. This enabled early diagnosis and " possible cure by early treatment ". He regarded x-rays and radium as the treatment of choice for rodent ulcers and early skin carcinomata, giving the best prospect of relief with the best cosmetic effect. He had noticed the increase of malignant change in lupus scars since the introduction of x-ray treatment for lupus. Epilation by x-rays for tinea tonsurans had been successful in bringing about a rapid cure, and had reduced the disease to negligible proportions. He felt at that time that x-ray therapy was one of the great advances in dermatology.

In 1931 he was president of the British Association of Dermatology meeting in Bristol. The next year he was made Sheriff of Bristol, and 5 years later became a magistrate. He was very interested in the Bristol Children's Hospital, and was president from 1934 to 1940. Another interest was the Y.M.C.A. Bristol branch, of which he was also president.

He retired in 1932, and was made Consulting Physician to the Skin Department.

John Alexander Nixon (1885–1964) was appointed Assistant Physician in 1906, and full Physician and Physician-in-charge of Dermatological Cases at the Royal Infirmary 2 years later, relinquishing the latter appointment when he became Professor of Medicine in 1924. He trained at Cambridge and St Bartholomew's Hospital, where he was president of the Abernethian Society and editor of the Hospital Journal. He was a most prolific writer, especially on topical medical subjects of his time, such as liver treatment of pernicious anaemia, insulin therapy, Salvarsan for syphilis, war injuries and illnesses. He published with his wife a

FIG. 5.—John Alexander Nixon 1885–1964.

" Textbook of Nutrition " (Nixon and Nixon, 1938). In all he had more than 50 papers accepted for publication.

Nixon, in 1915, described *Pediculosus ventricosus* as the cause of cotton-seed dermatitis. He investigated outbreaks in Bristol and Avonmouth docks, where the dockers were bitten on the arms and neck. He noted that the mite does not become parasitic on man, as none were bitten afresh; nor were the families affected (Nixon, 1915).

During the First World War he was promoted to temporary colonel and awarded the C.M.G. for his work in France and Germany. He was president of the Bristol Medico-Chirurgical Society in 1922, when he chose as the subject of his address—" The Debt of Medicine to the Fine Arts " (Nixon, 1923) and later published a book with the same title (Nixon, 1938).

In 1930 he was president of the Bath and Bristol branch of the British Medical Association, and in the same year he gave the Long Fox Memorial Lecture.

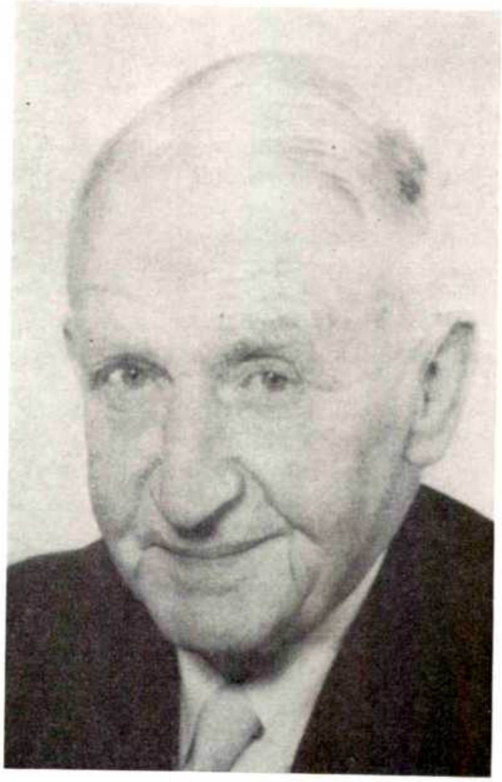

FIG. 6.—Stuart Hardy Kingston 1885–1964.

Stuart Hardy Kingston (1885–1964) qualified in Bristol in 1910, and took his D.P.H. in 1920. He succeeded Professor J. A. Nixon in 1924 as Dermatologist at the Bristol Royal Infirmary. He had previously held posts as Honorary Clinical Assistant to the Skin, Syphilis and Light Therapy Department of the London Hospital, and also to the Skin Department of the Children's Hospital, Great Ormond Street, as well as St John's Hospital for Diseases of the Skin in London. He was interested in venereal diseases and was appointed Director of the Venereal Diseases Department of the Infirmary. He was Consulting Dermatologist to the Ministry of Pensions in Bristol.

Norman Francis Clifford Burgess (1902–1940) was appointed as Assistant Physician to the Skin Department at the General Hospital in 1929, and Physician to the Department when Dr Wills retired in 1932. He was also Dermatologist to Southmead, Cossham and the Homoeopathic Hospitals. He trained at Cambridge, and entered Guy's Hospital as a senior science scholar, subsequently being appointed Paulton Research Student and Arthur Durham Travelling Student, which enabled him to visit Philadelphia, where he worked with Dr J. F. Schamberg, as well as Paris and Vienna. He obtained his M.R.C.P. in 1926, and 3 years later the M.D. Cantab. In 1931 he was awarded a B.M.A. research scholarship.

At the annual meeting of the British Association of Dermatology in 1927, he presented work done in Dr H. W. Barber's Department at Guy's Hospital on the study of calcium metabolism in 67 cases of skin disease, chiefly urticaria, Besnier's prurigo and erythema pernio (1928). The method was to estimate the total calcium in the ash of the serum, and by a direct precipitation process in the patients and controls. He found a normal figure for the total calcium, with a diminished precipitable calcium in most cases.

In 1931 he read a paper at the annual meeting of the British Association of Dermatology on auto-haemotherapy—a form of treatment in common use at that time, in which he tried to throw some light on its mode of action (Burgess, 1932). At the 9th International Congress of Dermatology in 1935 he spoke on the treatment of lupus vulgaris by intra-dermal injections into the lesions of phenyl-ethyl hydnocarpate, previously used for leprosy (Burgess, 1935).

FIG. 7.—Norman Francis Burgess 1902–1940.

Burgess had many interests. He was Bristol Police Surgeon, and a keen Territorial Army officer; he was also an energetic worker for the St John Ambulance Brigade, and became Commissioner for Bristol and had the Order of St John of Jerusalem conferred upon him.

Burgess died in 1940 at the early age of 38, and so served the Hospitals for only 11 years. During this time he published 23 articles, and from his student days was a brilliant scholar. His early death meant a very great loss to dermatology in Bristol.

Catherine Jane Maclaren was appointed temporary Junior Dermatologist in 1941—a post she held until the Author succeeded her as temporary Honorary Dermatologist in 1946. She continued with her appointments as Dermatologist to the Bristol Homoeopathic and Weston-Super-Mare Hospitals until she retired in 1963.

The Author and *Dr R. P. Warin* were both appointed Dermatologists to the United Bristol Hospitals and South Western Regional Hospital Board in 1948.

Dr R. R. M. Harman was appointed a Research Fellow to the United Bristol Hospitals, and Dermatologist to the South Western Regional Hospital Board in

1963. In 1969, at the end of his research fellowship, he became Dermatologist to the United Bristol Hospitals, as well as the Regional Board.

Dr R. D. G. Peachey was appointed Dermatologist to the United Bristol Hospitals in 1971, on the retirement of Dr C. D. Evans.

Gloucestershire

Dr E. N. Davey, a pathologist, treated skin diseases in Gloucester and *Dr R. Anderson*, a physician, those in Cheltenham before the appointment of a dermatologist in 1947.

Dr David Erskine was appointed Dermatologist to the North Gloucestershire Clinical Area in 1947. He left in 1950 to work in London. *Dr R. E. Bowers* succeeded Dr Erskine in the same year.

Somerset

Bath. Richard Nash (Beau Nash) was largely responsible for founding the Mineral Water Hospital in 1738. On the main staircase now hangs a painting by William Hoare, dated 1742, which shows a small boy with an eruption on his hands; he is being examined by Dr Oliver and Mr Peirce—an early joint consultation between a physician and a surgeon.

William White (1808) described an outbreak of scalp ringworm in Bath and the surrounding district. He noted that it affected the heads of children, particularly in boarding schools, was contagious, and could be caught from combs, hats and caps. It started as a small, red, irritating patch; then the hair fell, leaving a scaling area. He found that local applications of mercury, nitric acid and solutions of zinc had little effect, and that internal medicines were valueless. He recommended weekly shaving of the head.

John Cottle Spender (1801–1865) of Bath, in 1835, reviewed 1,000 cases of leg ulcers. He recommended removing the dressings as infrequently as possible, and also compression treatment from the toes to the knees (1835). Two years later he published a case of incipient gangrene of the big toe treated by firm bandaging, which reduced the oedema of the foot and lower leg, and prevented further gangrene by improving the circulation (1837).

John Kent Spender, his son, was a physician on the staff of the Mineral Water Hospital, and was chiefly interested in rheumatic diseases. He maintained that the Bath waters had a remarkable beneficial effect on a case of psoriasis, but advocated that arsenic by mouth should be given at the same time (1883). Dealing with epidemics of erysipelas in asylums and prisons, he found that a local application of tannin in spirit of wine and water in equal parts was helpful (1882a). He reported a patient with bronzing of the skin, looking rather like sunburn, and associated with debility and emaciation, as Addison's disease (1857).

He wrote a long article in the Association Medical Journal on the pathology and treatment of leg ulcers (1856). He agreed with his father that firm support was the essence of treatment; he pointed out that varicose veins were the common cause of leg ulcers which, at that time, was not always appreciated as the cause but thought to be concomitant. He later (1868) published a book on the same subject.

In his book on the Bath Thermal Waters (1882b) there is a section on diseases of the skin, and he mentions again the beneficial effect of the waters on psoriasis in 241 patients, treated at the Mineral Water Hospital between 1752 and 1764, although he states that treatment is very protracted. The condition at that time was called leprosy (Lepra Alphos of Erasmus Wilson).

He was awarded the Fothergillian gold medal of the Medical Society of London for his prize essay on " Therapeutic Means for the Relief of Pain " (1874).

The Bath Directory for 1866–67 lists the York Street Dispensary for " Diseases of the Skin, etc. " The Dispensary moved to Lower Church Street the next year, and the first surgeon-in-charge was *David Michael* (1831–1884). He studied at King's College Hospital, and then held the posts of House Physician and House Surgeon to the Royal United Hospital at Bath. He was elected F.R.C.S. in 1861.

He was succeeded in 1885 by *Henry Clifford Hopkins* until 1899, when *Richard Arthur Bayliss* was appointed; he appears to be the only member of the staff to publish an article on dermatology. He wrote about the Bath Mineral Water in the treatment of diseases of the skin (1902) from his experience as resident Medical Officer of the Mineral Water Hospital. He maintained that only certain chronic conditions, such as psoriasis, but not eczema, were suitable, and derived benefit from the warm water having a sedative effect on the peripheral nerves. The waters should also be taken internally to cause diuresis.

Charles Herbert Welch was in charge of the Dispensary from 1911 until it closed 2 years later.

The Royal United Hospital dates from 1826, and was a union of the Bath Pauper Charity (1747) and the Casualty Hospital (1788). The Hospital moved to the present site at Combe Park, 2 miles from the city centre, in 1926. The Manor Hospital, formerly an American Army Hospital, adjoins it and forms part of the group. St Martin's Hospital at Combe Down is a busy General Hospital, which was originally under the care of the Guardians.

Dr R. Edridge, a general practitioner, was interested in skin diseases and held clinics twice weekly at the Royal United Hospital. The *Author* was appointed Assistant Dermatologist in July 1938 and Dermatologist in 1946. *Dr J. H. Green* was appointed Dermatologist in 1946 to the Bath clinical area.

Taunton. No Physicians or Surgeons were known to take a special interest in diseases of the skin until *Dr J. H. Green* was appointed Dermatologist in 1948. He was succeeded by *Dr E. J. T. Prettejohn* in 1954.

Devon

Exeter. The hospital, now the Royal Devon and Exeter Hospital, was founded in 1741. Chemistry, as well as anatomy and surgery, was taught in Exeter from 1823 (Poynter, 1966). No record has been found of any physicians who specialized in treating skin diseases until the appointment of *Dr J. R. Simpson* in 1947.

Torquay. Dr A. Bowyer was appointed to the Torbay Hospital as Dermatologist in 1969.

Plymouth. Dr W. A. Lister, a general physician now retired, took a particular interest in diseases of the skin. He worked in Plymouth from 1926 until he was mobilized in 1939. During the War he was in charge of medical divisions of large hospitals and had under his care skin wards—up to 60 beds—in Egypt and India. In 1938 his paper on the natural history of strawberry naevi was of great interest (Lister, 1938). He mentioned that, although colleagues had said that these naevi disappear spontaneously, and that textbooks gave the same impression, no one had systematically studied the condition. He had carried out a 7-year follow-up of 77 patients with 93 naevi and found that all regressed, although they grew in the first 6 to 12 months of life. He maintained that no drastic measures were justifiable.

Dr F. A. Whitlock was appointed Dermatologist at Plymouth in 1948 until 1955, when *Dr R. D. Sweet* was appointed in his stead.

Cornwall

Dr C. T. Andrews and *Dr L. W. Hale*, both general physicians, at Truro and Redruth respectively, cared for patients with diseases of the skin.

Dr F. A. Whitlock was appointed as Dermatologist in 1948, and left in 1955, when *Dr Mark Hewitt* replaced him.

I am indebted to Librarians of the Bristol and Bath Reference Libraries, the Wellcome Institute of the History of Medicine, the University of Bristol Medical Library and the Royal College of Surgeons for their help. My thanks are due to the editor of the *Bristol Medico-Chirurgical Journal* for permission to reproduce the illustrations of Dr A. J. Harrison and Dr H. Waldo, and to Messrs Arrowsmith for the one of Dr W. Budd. The Medical Illustration Department of the University of Bristol copied the photographs for me.

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Obituary

JAMES GARLICK COBURN

Dr J. G. Coburn, Consultant Dermatologist at Rochdale, North Manchester, Booth Hall Children's Hospital and the Manchester & Salford Hospital for Skin Diseases, died suddenly on September 19th, 1971 whilst on holiday in Paris.

He was born in Glasgow on September 27th, 1917, and educated in Edinburgh and Lancaster before qualifying M.B., Ch.B. (Manc.) in 1942, having acquired a B.Sc. (Manc.) during his training. After a brief period as house physician at the Manchester Royal Infirmary he served in the R.A.F. until his demobilization in 1945. His interest in dermatology began during his war service and increased when he joined the staff of the Manchester & Salford Hospital for Skin Diseases on returning to civilian life. He proceeded M.D. (Manc.) in 1948.

Although primarily a clinician, he soon developed a special interest in histopathology and was encouraged and tutored by the late Dr Louis Savatard. For many years he was lecturer in dermatological histology at the University of Manchester.

He became consultant to various hospitals in the Manchester region and his particular interests, apart from histology, included tumours, leprosy, the treatment of acne with dermabrasion and the pathogenesis of eczema. An article on the last subject had just been completed before his death. He served as Secretary-Treasurer of the North of England Dermatological Society for 10 years and was elected President in 1963–64.

He had great personal charm, was an enthusiastic and discriminating collector of pictures, enjoyed good food and was a most generous and delightful host (with his wife) at their large house in Glossop.

He was devoted to his family and is survived by his wife (also medically qualified), a son (a doctor) and his two daughters.

G. Auckland

Br. J. Derm. (1972) **86**, 193.

Comment

COLLAGEN, COLLAGENASE AND CLINICIANS

The mechanisms by which collagen is degraded are fundamental to an understanding of orderly growth and remodelling, ageing, and pathological connective tissue resorption. Although this has been appreciated for many years, investigators have faced the paradox that native collagen is quite resistant to degradation by most proteolytic enzymes. *Clostridium histolyticum* produces a collagenase capable of degrading native collagen under physiological conditions (Mandl, 1961) but until 1962 no animal collagenase had been described. At that time Gross and Lapiere (1962) and Lapiere and Gross (1963) reported that in the medium of tissue cultures of metamorphosing tadpole tails a collagenolytic enzyme was present. Subsequently collagenase has been demonstrated in: human skin (Fullmer *et al.*, 1966; Eisen *et al.*, 1968, 1969a, 1970a, 1971; Lazarus and Fullmer, 1969; Bauer *et al.*, 1970), synovium from patients with rheumatoid arthritis (Evanson *et al.*, 1967, 1968; Lazarus *et al.*, 1968c; Harris *et al.*, 1970b), synovial fluid from patients with rheumatoid arthritis (Harris *et al.*, 1969b), human polymorphonuclear leukocytes (Lazarus *et al.*, 1968a, b; Daniels *et al.*, 1969, 1971), involuting post-partum rat uterus (Jeffrey and Gross, 1970), bone of various species (Walker *et al.*, 1964; Fullmer and Lazarus, 1967, 1969; and Shimizu *et al.*, 1969), human gingivae (Fullmer and Gibson, 1966; Fullmer *et al.*, 1969, 1970), crab hepatopancreas (Eisen and Jeffrey, 1969), human middle ear cholesteatoma (Abramson, 1969), cornea (Itoi *et al.*, 1969 Slansky *et al.*, 1969; Brown *et al.*, 1969), and Carageenin Granuloma (Perez Tamayo, 1970).

The methods employed in the study of collagenases depend upon the biochemical characteristics of collagen. Consequently, I will briefly review some of the more salient advances in collagen chemistry. Collagen is the major structural connective tissue macromolecule and it accounts for one-third of total human protein. Collagen is synthesized in fibroblastic cells by assembly of a proline rich polypeptide on ribosomal complexes, hydroxylation of appropriate proline and lysine residues to hydroxyproline and hydroxylysine, and glycosylation of some of the hydroxylysyl residues to galactosylhydroxylysine and glucosygalactosylhydroxlysine (Prockop, 1970). The collagen molecule may then be secreted as a precursor " Procollagen " which is rapidly converted by a proteolytic mechanism to tropocollagen molecules (Layman *et al.*, 1971; Bellamy and Bornstein, 1971). The tropocollagen molecules have been extensively studied and have a molecular weight of 300,000 and are 3000 Å in length and 15 Å in width (Piez, 1967). Tropocollagen molecules are comprised of 3 polypeptide chains each of 100,000 molecular weight. These chains are designated α and are characterized by the repeating gly-x-y triplet which is necessary for collagen helix formation. There are significant differences between constituent α chains so that in most collagens distinctive species are separable by chromatography (Piez, 1967; Miller, 1971a, b). These α chains may be crosslinked to give β components (2 α chains) and γ components (3 α chains). This intramolecular crosslink is formed by oxidative deamination of lysine by lysyl oxidase to form β-aminoadipic-semialdehyde (Pinnell and Martin, 1968) and it occurs at the lysyl residues at the fifth position from the A or N-terminal end of the molecule (Kang *et al.*, 1966). The crosslink area is further distinguished by a lack of polyproline helix (Piez, 1967), which may offer an explanation for the limited collagenolysis effected by certain proteolytic enzymes. Denaturation of collagen is the conversion of this rigidly coiled 3-chain molecule to a random coil called gelatin. The unique rope-like conformation of native collagen is responsible for its resistance to degradation by non-specific proteases; gelatin is rapidly destroyed by a variety of proteolytic enzymes.

The individual collagen molecules, secreted by the fibroblast, rapidly aggregate into fibrils so that the molecules lie parallel to each other and are displaced by one, two or three quarters of their length. The aggregation of molecules into this arrangement is controlled by electrostatic forces so that positively and negatively charged sites on adjacent tropocollagen molecules neutralize each other (Kühn *et al.*, 1970). Fibrils are further stabilized by a complex and as yet incompletely characterized system of covalent crosslinks based on a semialdehyde derived from lysine and hydroxylysine (Bailey *et al.*, 1970). Collagen fibrils are extremely stable and if they are not heated above their shrinkage temperature constituent collagen molecules are degraded only by true collagenases.

Segment long-spacing collagen (SLS) is an extremely valuable crystallite of collagen in which all of the collagen molecules are in register (Hodge, 1967). This allows visualization of the entire length of the molecule and therefore can be used to define the exact site of cleavage of collagen molecules by proteolytic enzymes.

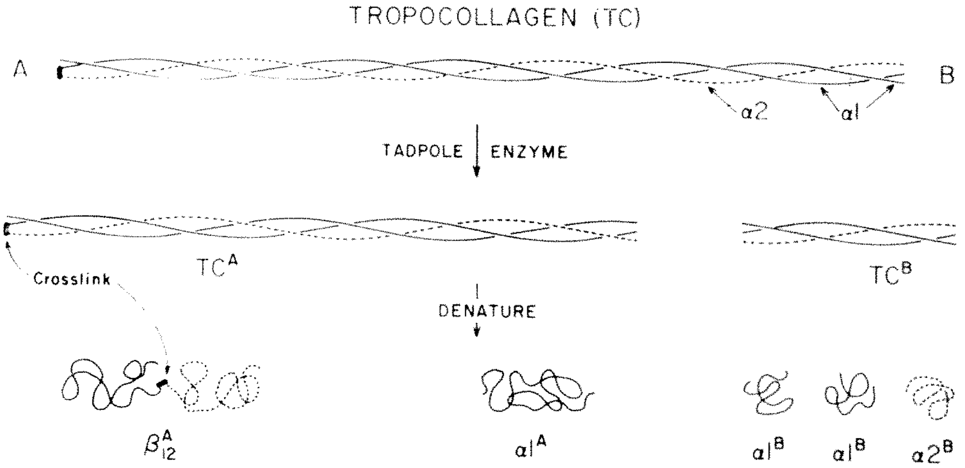

FIG. 1.—Diagrammatic representation of the collagen molecule and the pieces resulting from digestion with tadpole collagenase. The letters A and B refer to the designations employed in electron microscopy to distinguish the ends of the molecule. In a sample of extractable collagen some molecules would be crosslinked between α_1 and α_2 as shown, others between the two α_1 chains and some not at all. The denatured products would then include $\alpha_2{}^{A}$ and $\beta_{11}{}^{A}$ in addition to those shown (from Kang *et al.*, 1966).

Collagenases are enzymes capable of degrading native collagen fibrils under physiological conditions of temperature and pH or are enzymes which cleave native collagen molecules in solution through the characteristic helical part of the molecule. This definition precludes enzymes such as trypsin and pronase being included as collagenases since they effect a limited digestion of an end of the native collagen molecule. Scrupulous attention must be given to the preparation of a native collagen substrate since gelatin can be degraded by numerous proteases. Finally, the demonstration that an enzyme is capable of degrading synthetic collagen substrates is inadequate proof of its being a collagenase. Recent studies have shown that purified collagenases do not digest the synthetic collagen peptide and that associated non-collagenase peptidases are responsible for this phenomenon (Heidrich, 1969; Harper and Gross, 1970; Evanson, 1971).

The tadpole enzyme was the first collagenase described and its mechanism of action has been extensively studied (Gross and Nagai, 1965; Nagai *et al.*, 1966; Kang *et al.*, 1966; Sakai and Gross, 1967; Harper *et al.*, 1970a, b, 1971; Seifter and Harper, 1970). The mechanism of action of other animal collagenases appears to be similar to the tadpole enzyme. Tadpole collagenase, under physiological conditions of temperature and pH, specifically cleaves the collagen molecule three-quarters of the way from the N-terminal end (Fig. 1) (Nagai and Gross, 1965). The resulting products are designated

TC^A and TC^B and they maintain their helical conformation in solution at temperatures below 28°C. These products can be induced to form SLS aggregates and can be studied electron microscopically (Fig. 2) (Gross and Nagai, 1965) or they can be separated on acrylamide gel electrophoresis (Sakai and Gross, 1967). The TC^A and TC^B fragments are denatured at temperatures significantly lower than native tropocollagen but even more significant physiologically, they are more susceptible to degradation by trypsin (Sakai and Gross, 1967). This suggests that under physiological circumstances, the collagenase cleaves the collagen molecule and that the resultant fragments then spontaneously denature and are degraded by other non-specific tissue proteases.

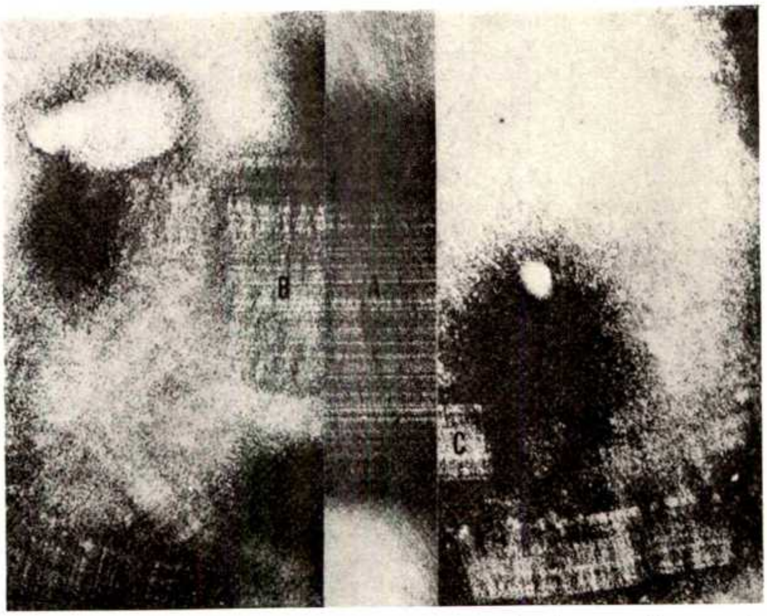

FIG. 2.—An electron microscopic composite photomicrograph of segment long spacing (SLS) units formed in a weak acetic acid solution from normal collagen (A), and collagen after digestion by collagenase derived from cultures of human gingivae. Note the 2200 Å SLS (B) representing the three-quarter length TC^A fragment and the 750 Å (C) which represents the one-quarter length TC^B fragment (Fullmer *et al.*, 1969).

The tadpole collagenase, like bacterial collagenase, requires calcium, is inhibited by EDTA and cysteine, and is unaffected by diisopropyl fluorophosphate. It should be emphasized that the bacterial collagenases in general, and the clostridial collagenase in particular, have a mechanism of action very different from the tadpole enzyme. The clostridial enzyme degrades collagen from both ends and generates a number of different sized peptides (Kühn and Eggl, 1966; Stark and Kühn, 1968).

Using an antiserum to highly purified tadpole collagenase immunologically reactive but enzymologically inactive collagenase has been detected in tadpole homogenates (Harper and Gross, 1970b; and Eisen *et al.*, 1971). The enzyme is in zymogen form and requires activation by a heat labile cofactor from collagenase-free tail-fin culture medium (Harper *et al.*, 1971).

Collagenase was initially detected in human skin by using collagen fibril culture plates (Fullmer *et al.*, 1966; Riley and Peacock, 1967). The enzymatic activity appeared 24 to 48 hr after incubation of the tissue and was produced primarily in the dermis (Eisen, 1969c; Lazarus and Fullmer, 1969). The skin collagenase is virtually identical to tadpole collagenase in its mechanism of action (Eisen *et al.*, 1968). The hydrolysis of collagen to TC^A and TC^B fragments by human skin collagenase occurs at neutral pH and is inhibited by EDTA, cysteine and human serum (Eisen *et al.*, 1968; Lazarus and Fullmer, 1969). The latter is probably mediated by the alpha$_1$-antitrypsin and alpha$_2$-macroglobulin of serum (Eisen *et al.*, 1970). These serum components appear to be capable of inhibiting all animal collagenases with the exception of the polymorpho-nuclear leukocyte collagenase (Lazarus *et al.*, 1968b) and the heavy synovial fluid collagenase (Harris *et al.*, 1969).

The human skin enzyme has been extensively purified by sephadex gel chromatography and an inhibiting precipitating antiserum prepared against it (Bauer *et al.*, 1970). This antibody to skin collagenase gives a reaction of complete identity on immunodiffusion with preparations of human gingival, rheumatoid synovial and granulocyte collagenase; it also completely inhibits the enzymatic activity of these human collagenases. Immunologically reactive but enzymologically inactive collagenase has been demonstrated in skin extracts using this antiserum (Eisen *et al.*, 1971). These inactive extracts have been chromatographed on sephadex gel and the alpha$_1$-antitrypsin and alpha$_2$-macroglobulin collagenase inhibitor separated from the enzyme. This procedure restored enzymatic activity to the immuno-reactive collagenase and strongly suggests that collagenase activity was masked in tissue extracts by binding of the enzyme to serum anti-proteases. Similar enzyme activation was obtained when enzymatically inactive culture medium from 6-hour old skin cultures was chromatographed. These data demonstrate that collagenase is present *in vivo* and therefore may have a significant role in the physiological remodelling of connective tissue.

Studies of dermolytic bullous dermatoses (dystrophic epidermolysis bullosa) suggest that collagenase may play a significant role in the pathophysiology of this disease (Eisen, 1969b; Lazarus, 1971a, b). This disease is characterized by sharp dermal–epidermal separation below the basement membrane which is associated with phagocytosis of collagen fibrils by macrophages and degeneration of connective tissue (Pearson, 1962). Cultures of normal appearing skin in this condition did not produce increased amounts of collagenase (Lazarus, 1971a) but the skin from lesions, which had a tendency to blister spontaneously, produced increased amounts of collagenase in culture (Eisen, 1969b; Lazarus, 1971a). These lesions were characterized by vascular proliferation, a perivascular round cell infiltrate and decreased amounts of stainable elastic tissue (Lazarus, 1971b). Rheumatoid synovium (Lazarus *et al.*, 1968c) and Carageenin granulomata have a similar histological picture and produce collagenase (Petez-Tamayo, 1970). These data suggest that vascular granulation tissue might be involved in collagenase production. In dermolytic bullous dermatoses it is not clear whether the granulation tissue itself, or the epidermis above it, is the source of collagenase. Although normal epidermis produces little collagenase by itself (Eisen, 1969a), epidermis over healing skin wounds and pure epidermis from a patient with dermolytic disease have been shown to produce significant amounts of collagenase (Eisen, 1969b; Eisen *et al.*, 1970; Donoff *et al.*, 1971). This might imply that an epidermal mesenchymal interaction is operative.

Another disease where collagenase has been implicated in pathological connective tissue resorption is rheumatoid arthritis (Evanson *et al.*, 1967, 1968; Lazarus *et al.*, 1968c; Harris *et al.*, 1970; Evanson, 1971). Collagenase has been found in the media from tissue cultures of rheumatoid synovium; this enzyme is quite similar to the skin collagenase. Enzyme production by cultures of rheumatoid synovium correlates both with systemic disease activity and the intensity of the rheumatoid process in the specific joint as estimated histopathologically (Lazarus *et al.*, 1968c). It should be emphasized that collagenase production is not unique to rheumatoid synovial cultures but also occurs in media from tissue cultures of other inflammatory synovia (Harris *et al.*, 1969b).

Synovial fluid from patients with rheumatoid arthritis, as a group, are less able to inhibit synovial collagenase than synovial fluids from patients with non-inflammatory joint disease (Harris *et al.*, 1969b) and in several cases directly assayable collagenolytic activity has been found in the joint fluid as well (Harris *et al.*, 1969b).

Degradation of collagen occurs during inflammation and abscess formation when significant numbers of polymorphonuclear leukocytes are present. This phenomenon is probably explained by the presence of an extractable collagenase in the granule fraction of polymorphonuclear leukocytes (Lazarus *et al.*, 1968a). This collagenase cleaves collagen molecules in solution so as to form the characteristic TC^A and TC^B products (Lazarus *et al.*, 1968b). The enzyme is operative at neutral pH and is inhibited by EDTA, and free sulphhydryl compounds such as cysteine (Lazarus *et al.*, 1968b; and Daniels *et al.*, 1969, 1971). The granulocyte collagenase is distinctive in that it is not inhibited by serum and requires the presence of other leukocyte proteases to effect collagen fibril degradation (Lazarus *et al.*, 1968b).

Degradation of the collagenous matrix is an extremely complex phenomenon. It is unquestionably a multi-step phenomenon with both extracellular and intracellular components (Woessner, 1971; Lazarus, 1971c). The secretion of lysosomal proteases such as cathepsin D (Dingle *et al.*, 1971) are also involved in connective tissue degradation. However, it is clear that the collagenases are of central importance in connective tissue degradation and are essential to an understanding of connective tissue catabolism.

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IRISH DERMATOLOGICAL SOCIETY

(Chairman—Dr J. Martin Beare)

Belfast, October 2nd, 1970

Porphyria Cutanea Tarda Treated with Sodium Bicarbonate.—Dr D. Burrows.

A woman aged 62.

History.—She developed blistering, friability and increased fragility of the skin 5 years ago after 3 anaesthetics over a period of 6 months for needling cataract. Anaesthetics consisted of pethidine, thiopentone, nitrous oxide and halothene. There was no sign of sensitivity reaction to halothene at the time. There is no history of jaundice or intake of alcohol. A diagnosis of porphyria cutanea tarda was made at that time. Her skin condition has gradually become worse and is particularly bothersome in the summer so that for instance she cannot garden because of the effect of sunlight and minor trauma on the skin.

Investigations.—(February, 1971.) Serum; glutamic oxaloacetic transaminase 164 K units/ml., pseudocholinesterase 68 units/ml., glutamic pyruvic transaminase 145 units/ml., lactic dehydrogenase 158 WL units, Bilirubin 1·1 mg./100 ml., Alkaline phosphatase 11 K units/ml., total protein 7·1 g/100 ml., iron 238 μg/100 ml., T.I.B.C. 255 μg/100 ml. Urine; uroporphyrin 1138 μg/24 hr, coproporphyrin 478 μg/24 hr.

Treatment.—In February she was started on slightly over 2 g sodium bicarbonate daily—enough to get her urine between pH 7 and 8. This summer she has had no trouble with her skin, although the weather has been sunnier than usual.

Comment.—Wiegand *et al.* (1969) suggested using sodium bicarbonate for porphyria cutanea tarda. The theoretical consideration for employing such therapy was that the production of an alkaline urine prevented diffusion of coproporphyrin back through the kidney tubule, thereby producing a selective excretion of increased amounts of coproporphyrin in the urine. As a consequence of this depletion of coproporphyrin, it was postulated that porphyrin precursors would be diverted from the uroporphyrinogen-uroporphyrin pathway toward coproporphyrinogen-coproporphyrin synthesis. It was hoped that the shunting of porphyrin synthesis toward coproporphyrin ultimately would decrease the circulating levels or uroporphyrin, the characteristic pathological porphyrin in porphyria cutanea tarda. Our preliminary studies indicated that lower levels of urinary uroporphyrins could be achieved by metabolic alkalinization and that eventually urinary excretion of coproporphyrin also would decrease. Perry *et al.* (1970) subsequently confirmed this. It showed that the treatment was most effective if patients gave up alcohol and was not so effective in those which continued their alcohol consumption. This patient is interesting in that her porphyria cutanea tarda is not the result of alcohol and she is improving with this treatment.

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Dermatitis Herpetiformis; Improvement with Gluten-free Diet.—Dr D. Burrows.

A woman aged 28.

History.—She gave a history of swelling of her abdomen and diarrhoea at the age of 2 and a diagnosis of coeliac disease was made by her general practitioner. She was apparently treated with a series of injections until the age of 6. She cannot remember having any dietary restriction at this time and since then has had no diarrhoea. She has, however, felt tired at intervals, requiring treatment from her G.P. with several courses of oral iron. Eight years ago she developed dermatitis herpetiformis (confirmed by biopsy) with typical small, very itchy blisters on the shoulders and buttocks and has required dapsone ever since, taking about 50 mg. daily. On the many occasions on which she has tried to stop it the dermatitis herpetiformis has always recurred.

Investigations.—Hb. 10 g./100 ml. Protein and electrophoresis normal with the exception of slight dimunition in alpha$_2$ globulin. Biochemical screening including calcium, phosphorus and alkaline phosphatase were normal. Serum iron 30%. T.I.B.C. 450%. Serum magnesium 1·9 mg. % (normal 1·7–2·3). D-Xylose 23% (normal excretion 15%). Glucose tolerance test with fasting level of 84 mg/100 ml with a peak of 116 at 1 h. Three day faecal fat less than 10 g (normal less than 15). C^{14} fat absorption test peak of 6 × 10 to minus 4%). Jejunal biopsy—dissecting microscopy showed a completely flat mucosa typical of severe gluten enteropathy. Barium meal and follow through—there was slight thickening of the mucosal folds in the jejunum and a little flocculation in the lower jejunum consistent with a malabsorption syndrome. No localized lesion seen in the small bowel or ascending colon.

Treatment.—She was put on a gluten-free diet and dapsone was withdrawn. She has been free of any lesions of dermatitis herpetiformis since then.

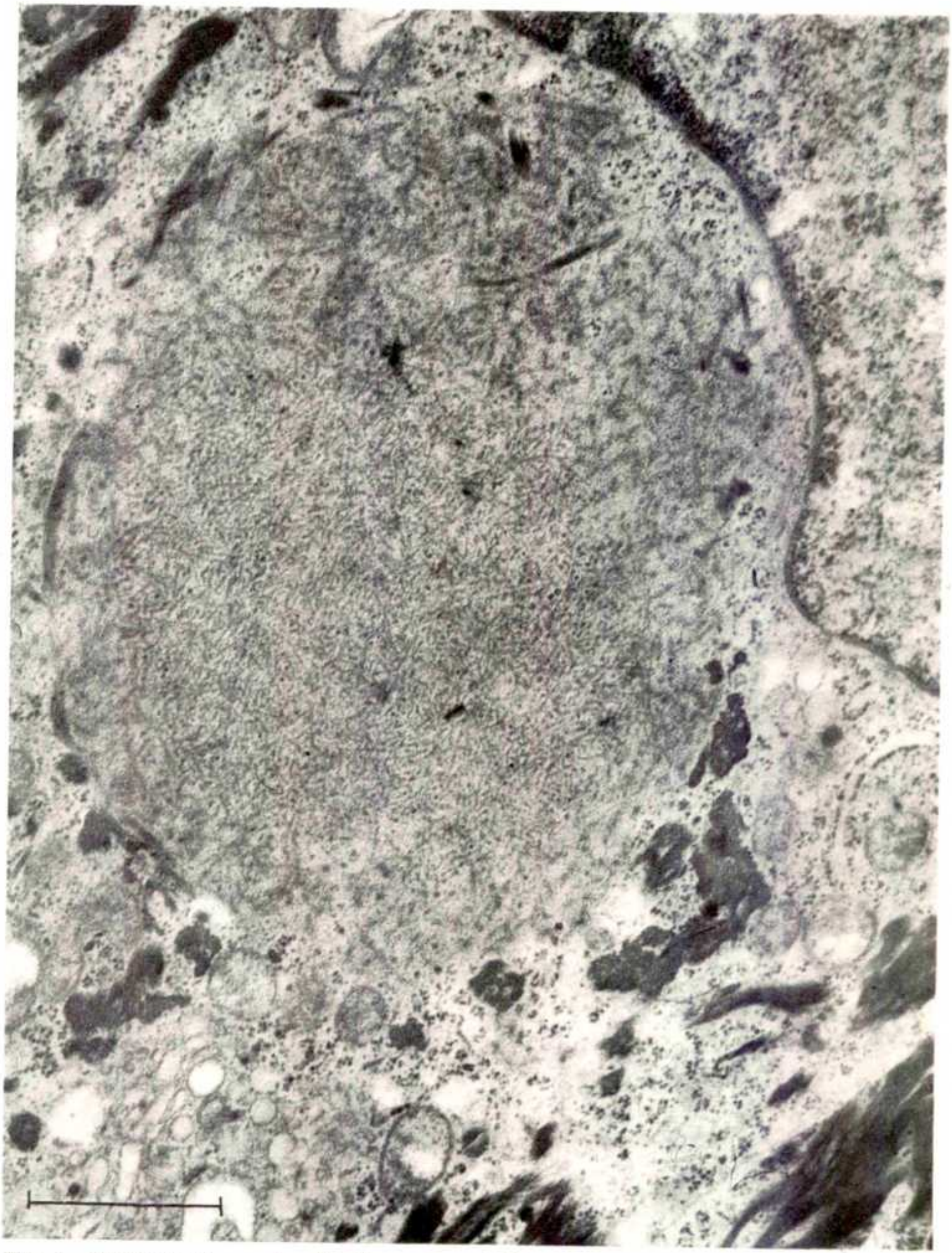

FIG. 1.—Electron micrograph. Fibrillae surrounded by microtubules, cytoplasmic organelles and tonofilaments are seen; nucleus in the upper right corner. Epon embedded uranyl acetate and lead citrate staining. ×22,000. |—| 1 micron.

Br. J. Derm. (1972) **86**, 203.

Correspondence

Macular Amyloidosis Cutis

Sir,—The macular form of localized amyloidosis cutis has become a matter of interest in the past few years and several recent reports include a considerable number of cases (Shanon and Sagher, 1970; Tay and Dacosta, 1970; Black and Wilson Jones, 1971).

We have observed one patient presenting the clinicopathological features of macular amyloidosis. The electron microscopic study confirmed the fibrillar (amyloid) nature of the dermal deposits staining as amyloid. In some instances, we found the same fibrillar material in the cytoplasm of keratinocytes. These deposits are surrounded by a peripheral zone of densely packed microtubules. Many other microtubules are seen sparsely disposed in the adjacent cytoplasm. The fibrillar deposits are not membrane limited, and thus cannot be phagolysosomal inclusions nor dermal pseudo-inclusions tangentially sectioned.

In their report of 21 cases, Black and Wilson Jones (1971) point out an histological feature perhaps indicating the presence of amyloid in the stratum malpighii: round hyaline bodies eventually containing a pycnotic nucleus and remnants of intercellular bridges. When such bodies were seen near the epidermodermal junction they stained as amyloid. We have not seen such intraepidermal bodies and the amyloid containing keratinocytes do not present degenerative features such as the hyaline body-like degenerating cell seen in light microscopy by Black and Wilson Jones.

However, our case seems to be the first report of the presence of amyloid in the cytoplasm of keratinocytes as proved by electron microscopy. The recent paper of Kurban *et al.* (1971), which is to our knowledge the only report of the electron microscopic study of macular amyloidosis cutis does not actually mention intra-cellular amyloid.

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The Action of Zinc Oxide on Dithranol

Sir,—Dr Dorothy Dean kindly showed us her letter (*Br. J. Derm.*, November 1971) and we can confirm her observation that the pink colour developing on mixing dithranol and zinc oxide is due to very small amounts of impurities in the zinc oxide, found even in the A.R. grade reagent. Our observation that zinc oxide (B.P.) removes dithranol from a paraffin solution such as hexane is a valid indicator of an incompatibility, which in actual practice impairs the therapeutic effect of dithranol in psoriasis.

Any implication that salicylic acid-free formulae should be satisfactory when pure zinc oxide and pure dithranol are used would be erroneous. The cost of purification of zinc oxide far outweighs the cost of salicylic acid; clinical efficacy would need to be established; stability parameters would be reduced. Nevertheless, at some centres, purification of dithranol by recrystallization from glacial acetic acid would improve therapeutic efficiency.

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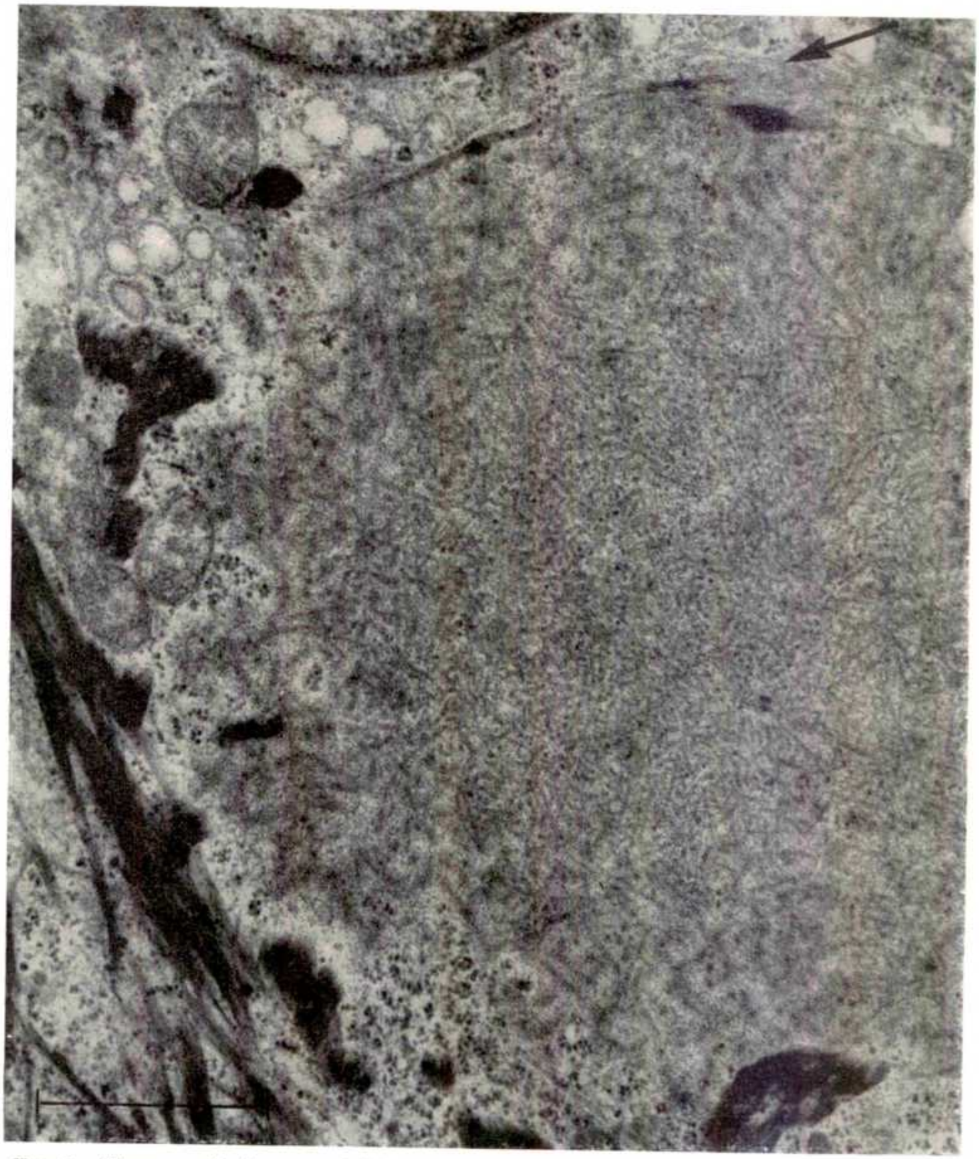

FIG. 2.—The same fibrillar material in another keratinocyte; microtubules in the dense zone surrounding the fibrillar deposit; sparsely disposed microtubules in the cytoplasm (arrow). ×25,000.

Zinc and Copper in Psoriasis

Sir,—We have completed the estimation of serum levels of zinc and copper in a new series of 52 psoriatic patients, using neutron activation analysis which is now accepted as the most sensitive, specific and accurate technique for trace element studies. We took account of area of skin involvement, duration of disease as well as type of therapy previously given. Estimations were also made in a control group of 40 healthy volunteers. None of the patients or control subjects was receiving oral contraceptives.

We could find no reduction in the mean zinc concentration in serum from the psoriatic group as compared with that of control, nor could we substantiate the findings by Greaves (1971) of a significant relationship between zinc levels and area of skin involvement (Table I). In addition, this study confirmed our previous findings in serum copper estimations in psoriasis, which we have found to be significantly increased (Molokhia and Portnoy, 1970). Again, there was no relationship between these increased levels and the extent of disease (Table II), which is in agreement with the findings of Kekki *et al.* (1966).

Table I.—*Zinc and Copper Levels in Serum of Psoriatic Patients and Normal Subjects*

Zinc levels (μg/g dry weight)

Type	No. of samples	Range	Median	Mean	S.D. (±)
Normal	39	5·79–15·60	10·20	10·50	2·01
Psoriasis					
Severe (10%)	18	7·20–18·70	10·50	10·75	2·52
Minor (5%)	32	7·05–15·60	10·20	10·25	2·07
Total	50	7·05–18·70	10·35	10·44	2·15

Table II.—*Copper Levels (μg/g dry weight)*

Type	No. of samples	Range	Median	Mean	S.D. (±)
Normal	40	7·54–13·80	10·10	10·40	1·50
Psoriasis					
Severe (10%)	19	10·40–18·40	14·10	13·85	2·76
Minor (5%)	33	8·63–23·00	12·40	13·45	3·19
Total	52	8·63–23·00	13·00	13·56	2·88

In the difficult task of quantitating the psoriatic state, the area of skin involvement is perhaps the most obvious and easily assessed factor, but there are others such as the intensity and number of individual lesions (Aschheim, 1969). Like Greaves (1971) we have once more found a wide range of zinc values in psoriatic serum, but we have shown that this is not due to variations in the area of lesions. The same wide range has been noted in serum copper levels (Table II). These wide ranges probably represent a deviation from the normal mechanism(s) responsible for the control of trace elements in the blood.

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Effects of Propranolol on the Acetylcholine Induced Response of the Human Sweat Gland

SIR,—We should like to make some comments on the article recently published in this journal, " Effects of Propranolol on the Response of Human Eccrine Sweat Glands to Acetylcholine " by Foster *et al.* (1971).

In this article the authors deny the effect of propranolol on the sweat gland response to acetylcholine as reported by Hemels (1970). However, from Table Ia (Foster *et al.*, 1971) it can be seen that, in 12 of the 14 tests carried out on 6 subjects the total output of sweat increased after beta blockade with propranolol.

These data are in accordance with the data reported by Hemels: a beta blockade resulted in an increase in sweat response (in mg.cm^{-2}). From the same table (Foster *et al.*, 1971; Table Ia) it can also be seen that the percentage increase in volume does not correlate with the heights of the responses. In addition to this, Table Ib presents no information about changes in volume.

However, we wonder what results would have been found, if water loss measurements had been carried out with a water analyser which gave a linear response to the amount of sweat produced. The dose response curves constructed earlier by Foster (1971) differ from the dose response curves obtained by Thiele and Hemels (1971) after stimulation of the human sweat gland by acetylcholine. With our measuring techniques, we find an increase in height of the responses with increasing doses of acetylcholine up to 0·1 mg, in contradiction to Foster's measuring technique, which shows a decrease in the height of the response with increasing amounts of sweat. We are currently preparing an account of a thorough comparison of acetylcholine induced water loss and carbon dioxide release measurements (Thiele and Hemels, 1971).

The argument that an intravenous dose of propranolol has no effect on the cat's footpad sweat gland response on plantar nerve stimulation seems to us of doubtful significance for the interpretation of the responses of human sweat glands on intradermal injections of acetylcholine: we wonder whether the Fig. 4 (Foster *et al.*, 1971) represents a blood pressure lowering effect of this drug.

In addition to this, we want to underline that our experiments were carried out in the months October and November. It appeared that during the summer the response of the sweat glands to a dose of acetylcholine equals the response to the same dose acetylcholine + propranolol in winter time. On the basis of the differences in seasonal sweat response to stimulation with acetylcholine, we assumed that beta adrenergic structures may be involved.

This statement does not imply that we understand the precise mechanism by which this occurs. An important factor could be the temperature of the skin, which in turn is related to the peripheral circulation, since skin temperature plays an important role in the reactions of the sweat glands (Hardy *et al.*, 1970; Thiele *et al.*, 1971; Thiele and Malten, 1971; Malten and Thiele, 1971; Nadel *et al.*, 1971).

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Candida Albicans Infection and Napkin Rash

Sir.—Brookes *et al.* (1971) isolated *Candida albicans* in small quantities from only 2 of 25 infants with napkin rashes who were attending the local authority health clinic. We were able to culture the organism in varying quantities from 48 (41%) of 117 infants similarly affected (Dixon *et al.*, 1969). It was suggested that the reason for the difference between their findings and ours is that we included in our series a number of children seen in hospital outpatient clinics who were more likely to have long-standing severe eruptions. However, 72 of our cases were collected during routine local authority clinics in the same way as theirs, and *Candida albicans* was isolated from the napkin eruptions of 30 (42%) of these infants. The napkin eruptions of the children in this group were of varying severity and length of history, and *Candida albicans* was most likely to be isolated from the more severe eruptions of longer standing. However, we were still able to culture the organism from the lesions of 12 (35%) of the 34 children from health clinics who had suffered from mild eruptions for less than 2 weeks.

If the discrepancy between the 2 findings is not due to difference in the selection of cases, or in the laboratory methods used, it may result from different scraping techniques. We used a blunt tenotomy knife instead of a scalpel blade. Because of its bluntness, this could be wielded vigorously on a baby's bottom to collect scrapings from various sites involved in the eruption within the napkin area. The scales were inoculated directly on to plates of glucose-peptone agar, and in many instances the blade was also cut into the culture medium. Brookes *et al.* sampled one previously specified site only.

Candida albicans can often be isolated from the alimentary tract of infants. In our study reported in 1969 we were able to culture the yeast from the faeces of 31 (43%) of the 72 infants with napkin rashes and of 11 (16%) of 68 normal infants who were attending child health clinics. In a more recent investigation, the results of which are as yet unpublished, we isolated *Candida albicans* from the faeces of 85 (39%) of 217 infants who were found by health visitors to have a napkin rash. In view of the wide distribution and opportunist nature of *Candida albicans*, it would be surprising if it were not frequently possible to culture the organism from napkin eruptions of all types. Although primary infection may sometimes occur, the organism is in most cases probably a secondary invader. As such, it may be a transitory inhabitant of little or no significance, or it may make the eruption much worse and delay its cure. In assessing the importance of *Candida albicans* infection in any particular napkin rash both clinical and mycological findings must be taken into account.

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Books

Human Hair Growth in Health and Disease. DAVID FERRIMAN (1971). Springfield: Thomas. Pp. 62, 12 illus. Price £2.50.

This small monograph is an account of the normal morphology and physiology of hair growth in the human, and the influence on these of endocrine abnormalities and other diseases. Mythology is ignored, the information contained in over 200 references is compressed within 50 pages and most of the work referred to has been published in the last decade.

A chapter reflects the author's interest in hirsutism in the female and in androgens and hair growth, and in an appendix, his own work in a semi-quantitative method of assessing the degree of terminal body hair growth.

Hirsutism is classified as primary and secondary and the account of the latter and its differential diagnosis is clear and concise. Some indication of the relative incidence of the diseases would have been helpful. Primary hirsutism is regarded from the premise that measures of testosterone production are increased in this condition, the sources being the adrenal cortex or polycystic ovaries. It seems to the reviewer that such an approach is being overtaken by events and that the local factor of hair follicle sensitivity to circulating androgens, mediated by cyclic AMP, is the more likely answer to the majority of cases, though in a book of facts such an hypothesis must necessarily receive only brief mention.

The chapter on alopecia relates most of the established facts but more space could have been devoted to pattern baldness in the female, in which incidentally it is untrue to say that marked temporal recession occurs.

I strongly recommend this book for its brevity, readability and for continuing to move the study of hair into the realms of science.

RONALD CHURCH

Symposium on Pediatric Dermatology. Ed. A. H. JACOBS (1971). Pediatric Clinics of North America, vol. 18, no. 3. Philadelphia: Saunders. Pp. 683–1008, illustrated. Price £2.50.

The Pediatric Clinics of North America, published quarterly, enjoy and deserve a high reputation. Few of the 70 or more volumes have been devoted to dermatology, although the editor of the latest addition to the series tells us in his introduction that " the primary or secondary complaint was referable to the skin in 34% of 500 children seen consecutively in routine paediatric practice ". Eleven of the 12 reviews in this volume present advances in dermatology for the benefit of practising paediatricians. The authors are dermatologists, or paediatricians and dermatologists writing in collaboration.

The first chapter, on topical therapy, is useful in bringing to the notice of dermatologists in Europe, some applications and procedures not widely used outside the United States, but covers so much ground that it offers too little positive guidance for the non-dermatologist. The chapter by Hodgman, Freedman and Levan on neonatal dermatology is good and is obviously based on extensive personal experience. Watson and Cann, writing on genetic counselling, have also contributed a useful chapter. Margileth, in an otherwise well informed chapter on developmental vascular abnormalities, rather surprisingly includes pyogenic granuloma as " a non-involuting telangiectatic nevus ".

The remaining chapters cover atopic dermatitis (Norins), contact dermatitis (E. Epstein), acne vulgaris (Arundell), psoriasis (Watson and Farber), pigmented nevi (Walton), connective tissue diseases (Tuffanelli and La Perriere) and disorders of the hair and scalp (Orentreich). All are competent and some are excellent. However, the recommendation of ammoniated mercury 2 to 5% for psoriasis is surely hard to justify.

Most of the chapters are clearly written but in some the language is at times clumsy and tortuous. It is not the duty of the editor of a scientific publication pedantically to impose on his contributors a uniform literary style, but he should perhaps intervene when an unhappy choice of words actually obscures an author's meaning.

There are 4 pages of illustrations in colour and large numbers of half-tone illustrations. The high quality of the former accentuates the poor definition of many of the latter, which have apparently been produced from colour transparencies, never a satisfactory procedure. The book is well designed and produced but there are many typographical errors.

ARTHUR ROOK

Br. J. Derm. (1972) **86**, 210

Quarterly Review

THERAPEUTICS XVII

"Reserve" Drugs in the Treatment of Tuberculosis

In this country the initial treatment of previously untreated tuberculosis, with the possible exception of lupus vulgaris, is still with the triple combination of streptomycin, para-aminosalicylic acid (PAS) and isoniazid. All other antituberculous drugs remain as "reserve" treatment; that is to say are indicated only when the organism is not sensitive to at least 2 of the standard drugs, where the toxic effects of 2 of the 3 are severe enough to contraindicate their use or the patient is unwilling to take them.

Primary resistance of *Mycobacterium tuberculosis* to one of the standard drugs is extremely low in this country. Miller *et al.* (1966b) found 3% of the mycobacteria resistant to one drug, 0·9% to 2 drugs and only 0·2% to all 3 drugs, a resistance level that had remained unaltered over several years (Fox *et al.*, 1957). The incidence of resistant organisms is higher in immigrants (Report, British Tuberculosis Association, 1966) and in patients with chronic, previously treated tuberculosis.

Hypersensitivity reactions are frequent with both PAS and streptomycin (Govindaraj and Grant, 1968). Toxic reactions are also well recognized, the most troublesome being gastro-intestinal disturbances with PAS, and vestibular damage with streptomycin. Peripheral neutritis occurring with isoniazid is, at least in part, determined by genetic differences in the rate of inactivation of this compound by acetylation (Clarke *et al.*, 1968).

There are now 13 "reserve" or newer drugs which have been developed for the treatment of tuberculosis. Clinical trials are continuing on their therapeutic effects, but 3 drugs appear important within the "reserve field". These are Rifampicin, Ethambutol and Thioacetazone.

Rifampicin

Rifampicin is the (3-(4 methyl)-1-piperazinyl-iminomethyl) derivative of Rifamycin S.V., produced by *Streptomyces mediterranei*.

Rifampicin is bactericidal (Dickinson and Mitchison, 1970). It has antibacterial activity against Gram-positive cocci comparable with penicillin, but is also active against Gram-negative bacteria particularly, *Haemophilus influenzae and Neisseria gonorrhoeae*. However, its greatest importance is its antimycobacterial activity. It is rapidly absorbed from the gastro-intestinal tract, peak serum levels are reached within 1 to 4 h and significant levels persist for up to 9 h (Canetti *et al.*, 1968). The drug is detoxicated by deacetylation in the liver. Approximately 10% is excreted in the urine, giving it a yellow colour; a useful guide as to whether the drug is being taken or not (Proust, 1971).

In vitro studies have shown a high level of susceptibility of human mycobacteria to Rifampicin, and a very low incidence of natural resistance even in wild strains (Verbist and Gyselen, 1968). At normal therapeutic dosage, serum concentrations up to 100 times the minimum inhibitory concentration for mycobacteria can be readily achieved (Canetti *et al.*, 1968).

Clinical studies have all confirmed the efficacy of Rifampicin as a potent antituberculous drug, and it has been successfully used as a "reserve" drug in the treatment of chronic tuberculosis where the organisms were resistant to all the standard drugs. Pines (1971) treated 26 such patients with Rifampicin 600 mg per day and Ethambutol 25 mg per kg per day in combination with one other "reserve" drug. Within 3 months 25 had negative sputum cultures.

Preliminary reports of Rifampicin in the initial treatment of previously untreated pulmonary tuberculosis are very promising. In combination with isoniazid or Ethambutol tubercle bacilli were eliminated from the sputum in 95–100% of patients within

20 weeks (Dormer and Salinger 1971; Newman *et al.*, 1971; Nitti *et al.*, 1971). Rifampicin and streptomycin in combination were slightly less effective. Mycobacterial resistance was not encountered when Rifampicin was given with either isoniazid or Ethambutol. In a small series of patients with lupus vulgaris treated with a large dose of Rifampicin a much more rapid cure was obtained than with ioniazid (Ayala, 1969).

The main toxic effect of Rifampicin is on the liver. It causes a transient rise in the transaminase levels and in some cases also a rise in serum bilirubin levels. However in most patients this is associated with only minimal symptoms and therapy can be continued often with a return of liver function tests to normal (Lees *et al.*, 1971; Proust, 1971; Nitti *et al.*, 1971 and Newman *et al.*, 1971). Lees *et al.* (1970a) reported severe jaundice in 4 out of 50 cases treated with Rifampicin. In 3 of these therapy was restarted without further jaundice, although the serum transaminase levels rose again. There is a particular risk in using this drug in patients who already have liver damage. Lesobre *et al.* (1969) reported 12 cases of jaundice with 4 deaths in 50 patients. Three of the 4 who died were chronic alcoholics and a similar finding was reported by Proust (1971). Liver function tests should be studied before starting Rifampicin and the drug avoided in patients known to be alcoholics, or to have had previous hepatitis.

Rifampicin is otherwise relatively non-toxic, but occasionally causes mild dyspepsia and hypersensitivity reactions. A transient skin rash occurred in a small number of patients treated with Rifampicin and isoniazid. In one patient this was severe enough to warrant change of therapy (Lees *et al.*, 1971).

Rifampicin is a very potent antituberculous drug but is very expensive. A year's treatment with Rifampicin and isoniazid would cost approximately £154 as compared with £23 for a year's treatment with the " standard " drugs. However, it is argued that the greater efficiency of Rifampicin justifies this and might ultimately lead to a shortening of either the time spent in hospital or the duration of treatment.

Earlier hopes of this drug being suitable for high dose intermittent therapy have now been dashed by the high incidence of side effects produced (Poole *et al.*, 1971).

Other uses of Rifampicin.—Although effective against Gram-positive and negative organisms, resistance quickly develops (Phillips, 1971). In view of its high anti-mycobacterial property it should probably be reserved for the treatment of tuberculosis and occasional serious staphylcoccal infections resistant to conventional drugs. Preliminary studies of Rifampicin in the treatment of leprosy are very exciting. The drug is bactericidal and kills the mycobacteria more rapidly than dapsone (Rees *et al.*, 1971).

Ethambutol

Ethambutol (2,2′-(ethylene diimino)-di-1-butanol dihydrochloride). This drug has a completely different structure from any other known anti-tuberculous agent and is effective only against mycobacteria. It is bactericidal. Ethambutol is rapidly absorbed after oral administration; peak blood levels are reached within 2 to 4 h (Place *et al.*, 1966). The *in vitro* anti-tuberculous effect was established by Karlson in 1961. Its value in the treatment of tuberculosis in experimental animals was demonstrated by Schmidt (1966). Resistant strains of mycobacteria quickly emerged if Ethambutol was given alone, but combination with isoniazid prevented this and enhanced the therapeutic effect. Clinical trials have confirmed the therapeutic efficacy of Ethambutol. A daily dose of 25 mg per kg combined with isoniazid 300 mg in the treatment of primary tuberculosis was as effective or marginally superior to standard PAS and isoniazid therapy (Donomae and Yamamoto, 1966; Bobrowitz, 1966). It was also more acceptable to the patients than PAS. Lower doses than 15 mg per kg are less effective (Fereebee *et al.*, 1966). In combination with other " reserve " drugs it has a very potent therapeutic effect in chronic tuberculosis. Lees *et al.* (1970b) used it in combination with a variety of " reserve " drugs in 36 patients with chronic tuberculosis resistant to all the standard drugs. Within 6 months 32 had negative sputum cultures. These authors comment on the lack of toxic effects. Macotela and Ruiz (1970) report 3 patients with cutaneous tuberculosis who were only cured when Ethambutol was added to their anti-tuberculous treatment.

The main toxic effects are on the eye. The lesion is a retrobulbar neuritis. This may be central leading to a reduction in visual acuity and loss of the ability to see green. A periaxial change gives rise to a defect of the peripheral visual field (Leibold, 1966). The toxic effects are related to the dose. Recovery of vision usually occurs but may take several months.

Other toxic manifestations are extremely rare and it does not appear to be hepatotoxic.

Ethambutol is a potent oral anti-tuberculous drug, and is particularly valuable in patients who cannot take PAS or whose organisms are insensitive to it (Fox, 1970). In more recent studies it has also proved to be an effective companion drug to Rifampicin, both in the treatment of primary and chronic tuberculosis (Pines, 1971). This too is an expensive drug. A year's treatment with this drug alone would cost £74. This expense may be justified in that some patients would take regularly an acceptable drug who would fail to take PAS.

Thioacetazone

This oral anti-tuberculous drug was first introduced and found to be therapeutically effective in Germany in the late 1940s. However it produced many side effects, including skin rashes, blood dyscrasias and liver damage and was thus little used (Hinshaw and McDermott, 1950).

In East Africa it was desirable to find a cheaper though equally effective alternative to PAS for use in combination with isoniazid. In the last 10 years the East African and British Medical Research Councils have conducted several clinical trials of the efficacy of Thioacetazone in combination with isoniazid. In a dose of 150 mg per day it is equally as effective as PAS and more acceptable to the patients. Further trials have shown that if streptomycin 1 g per day is given in addition for the first 8 weeks of treatment, the therapeutic effects are enhanced (East African and British Medical Research Councils, 1960, 1963, 1966 and 1970).

The toxic effects in the East African studies were surprisingly low and limited to 5% of the patients treated. Skin rashes occurred, many of which were mild, occasional liver damage and mild gastro-intestinal symptoms. This compared favourably with the toxicity of PAS. As a much higher incidence of toxic reaction had been reported elsewhere an international survey was undertaken by the M.R.C. and the Chest Disease Research Unit (Miller *et al.*, 1966b). Two thousand patients were involved from 13 countries. All were treated with streptomycin and isoniazid in conventional doses. Half the patients received in addition 150 mg of Thioacetazone. The overall incidence of side effects was 20·4% for those receiving Thioacetazone and 7·8% for those not on the drug. The incidence of side effects in the different countries was very variable, being low in Kenya, high in Nigeria and highest in both Hong Kong and Czechoslovakia.

The Hong Kong and British Medical Research Councils (1968) confirmed the high incidence of toxicity to Thioacetazone in combination with isoniazid. Skin changes occurred in 37% of the patients treated and included generalized eruptions, pigmentation, hair loss and stomatitis. Exfoliative dermatitis was not observed. In Britain, Pines (1964) reported severe toxic reactions in 6 out of 11 patients treated with these two drugs.

This wide variation in the incidence of toxicity in different parts of the world is of theoretical interest, but of practical importance. Possible explanations include pharmacogenetic or racial differences, environmental factors both natural and related to the supervision of treatment, and previous medication.

Thioacetazone is a very cheap drug. A year's treatment with this drug and isoniazid would be approximately £2·50, as compared with £23 for a year's treatment with isoniazid and PAS. It follows that Thioacetazine has a valuable place in the primary treatment of tuberculosis in areas of the world where economic factors necessitate the use of the cheapest drug, provided that this area is one in which the incidence of toxic effects is low.

Other effective anti-tuberculous drugs include Cycloserine, Pyrazinamide, Ethionamide, Prothionamide, Capreomycin, Kanamycin, Viomycin, Gentamycin and Thiocarlide. These drugs, however, in contrast to the 3 compounds described at length, are unlikely on the grounds of economy, patient acceptability or potency to achieve a place as " first line " drugs in the treatment of tuberculosis.

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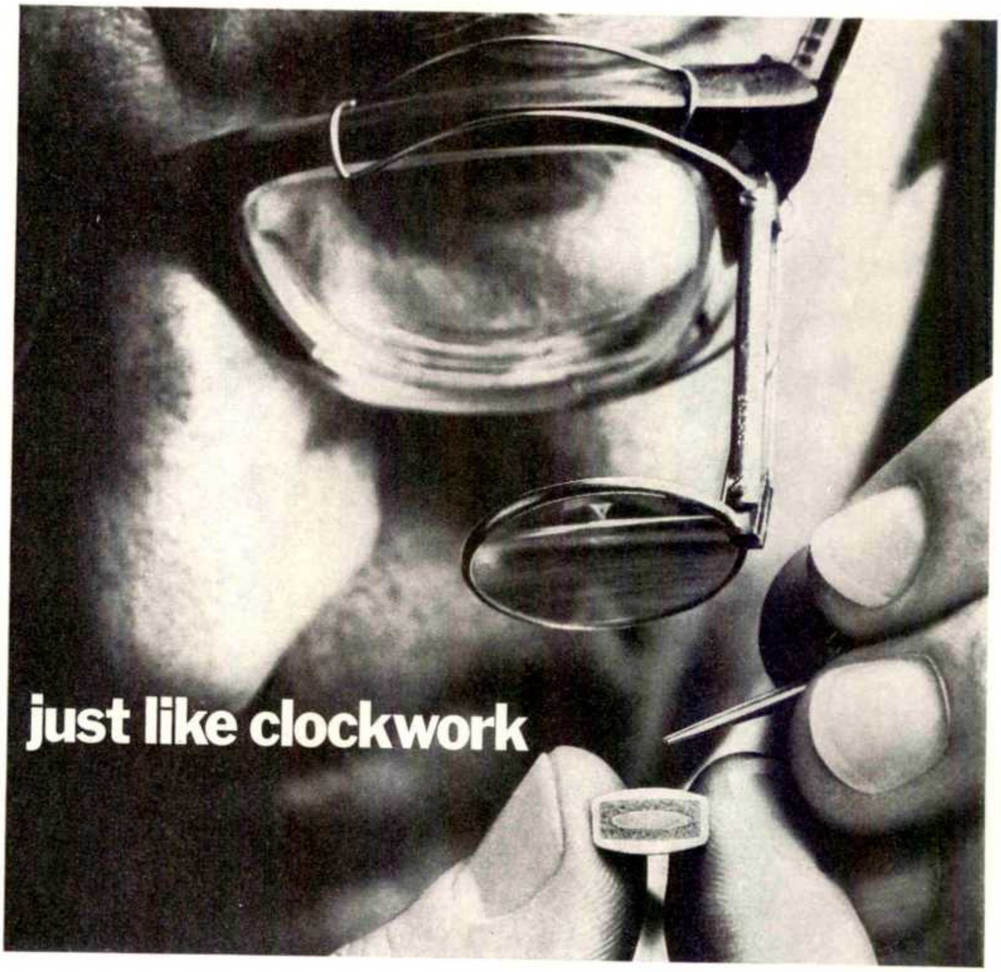

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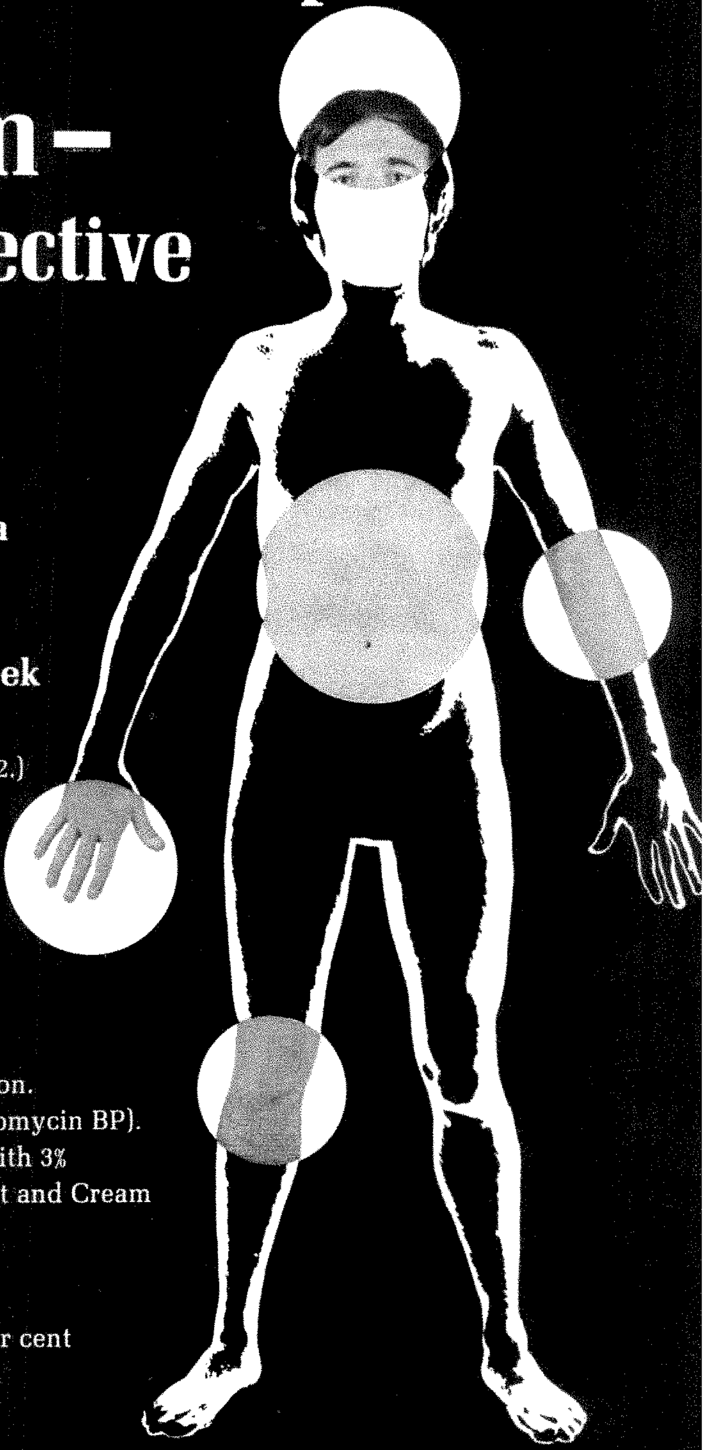

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CONTENTS (*continued*)

Obituary

JAMES GARLICK COBURN 192

Comment

Collagen, Collagenase and Clinicians
GERALD S. LAZARUS 193

Society Proceedings

Irish Dermatological Society, October 2nd, 1970 200

Correspondence

Macular Amyloidosis Cutis
J. REVUZ, J. POIRIER and R. TOURAINE 202
The Action of Zinc Oxide on Dithranol
STANLEY COMAISH and JOHN SMITH 202
Zinc and Copper in Psoriasis
BENJAMIN PORTNOY and MOHAMED M. MOLOKHIA 205
Effects of Propranolol on the Acetylcholine Induced Response of the Human Sweat Gland
H. G. W. M. HEMELS, F. A. J. THIELE and F. W. BAUER . . . 206
Candida Albicans Infection and Napkin Rash .
P. N. DIXON, M. P. ENGLISH and R. P. WARIN 207

Books

Human Hair Growth in Health and Disease (Ferriman); Symposium on Pediatric Dermatology (Jacobs, (Edit.)) 208

Quarterly Review

Therapeutics XVII. " Reserve " Drugs in the Treatment of Tuberculosis
ANNE E. WALKER, E. FRENK and A. J. SMITH 210

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

Factors Contributing to Occasional Failures in the Laboratory Diagnosis of Bullous Pemphigoid by Indirect Immunofluorescence
Tadeusz P. Chorzelski and Ernst H. Beutner 111
Demonstration of Intercellular Immunofluorescence and Epidermal Hysteresis in Bullous Fixed Drug Eruption Due to Phenolphthalein
Walter B. Shelley, Otto L. A. Schlappner and Harold B. Heiss 118
The Glands of Montgomery
William Montagna and Jeung Soon Yun 126
The Activity of Metabolic Enzymes of Human Epidermis in Relation to Age
S. Yamasawa, D. Cerimele and F. Serri 134
Adenosine 3′ : 5′-Cyclic Monophosphate Phosphodiesterase in Skin. I. Measurement and Properties
P. D. Mier and E. Urselmann 141
Adenosine 3′ : 5′-Cyclic Monophosphate Phosphodiesterase in Skin. II. Levels in Atopic Dermatitis
S. W. J. Holla, E. P. M. J. Hollman, P. D. Mier, W. J. B. M. v.d. Staak, E. Urselmann and J. A. Warndorff 147
The Cutaneous Reactions Produced by *o*-Chlorobenzylidenemalononitrile and *ω*-Chloroacetophenone when Applied Directly to the Skin of Human Subjects
P. Holland and R. G. White 150
Cement Dermatitis and Atmospheric Parameters in Kuwait
M. Wajdi Kanan 155

Comparative Dermatology

Innervation of Sweat Glands in Horses and Dogs
Mary Bell and William Montagna 160
Generalized Cutaneous Eruption in a Dog, with Histological Similarity to Human Mycosis Fungoides
D. F. Kelly, R. E. W. Halliwell and R. M. Schwartzman . 164

Pharmacology and Treatment

Grenz Ray Treatment of Recurrent Herpes Simplex
A. G. Knight 172

Case Reports

Microsporum Equinum Infection of the Scalp in an Adult
K. J. O'Grady, M. P. English and R. P. Warin 175
Jadassohn Tumour Arising at Smallpox Vaccination Site
David Porter and James Earle 177

History of Dermatology

A History of Dermatology in Bristol and the West of England
Clifford D. Evans 180

(*Continued on* p. iii *of cover*)

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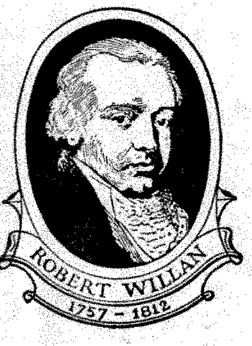

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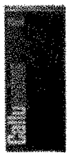

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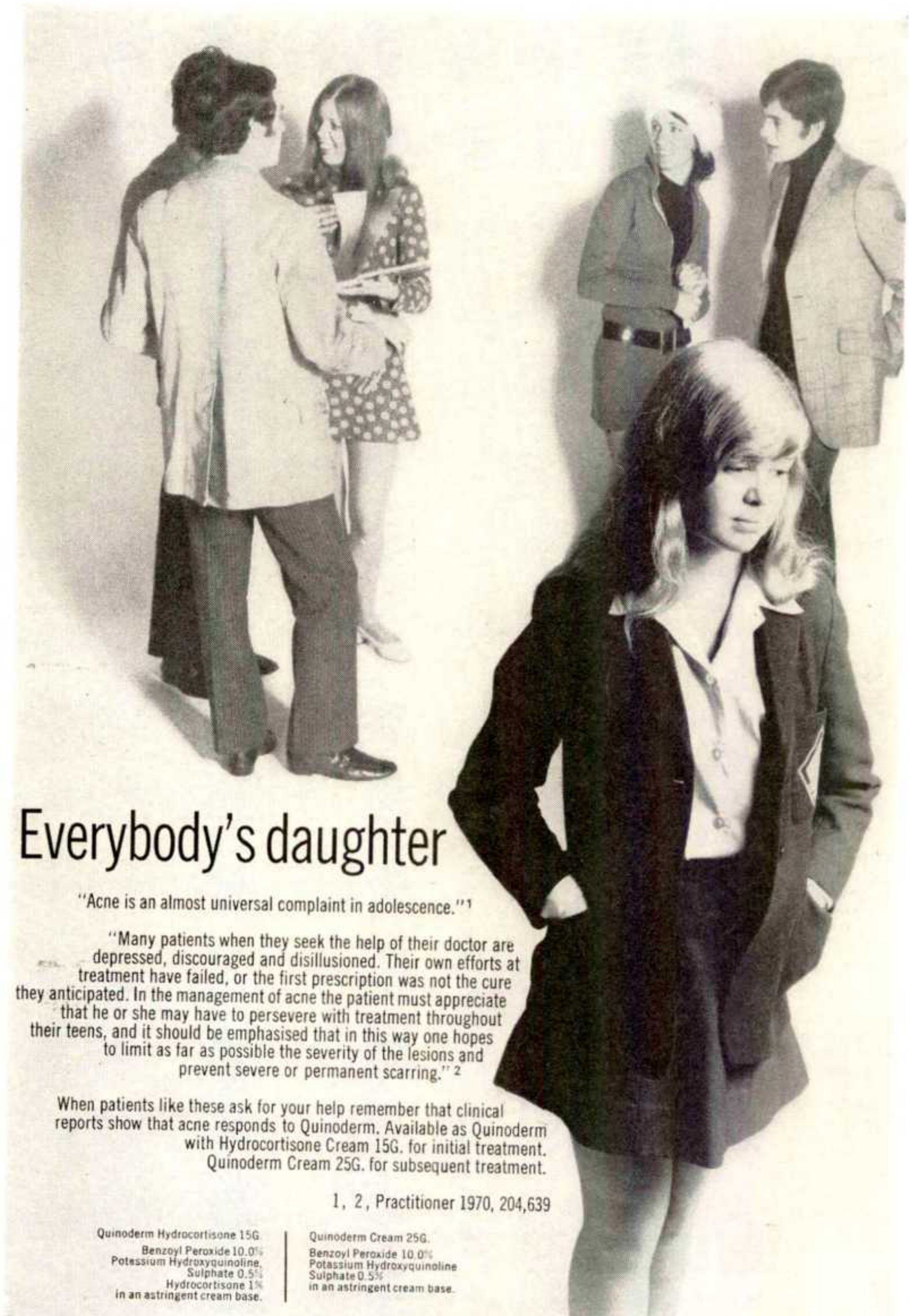

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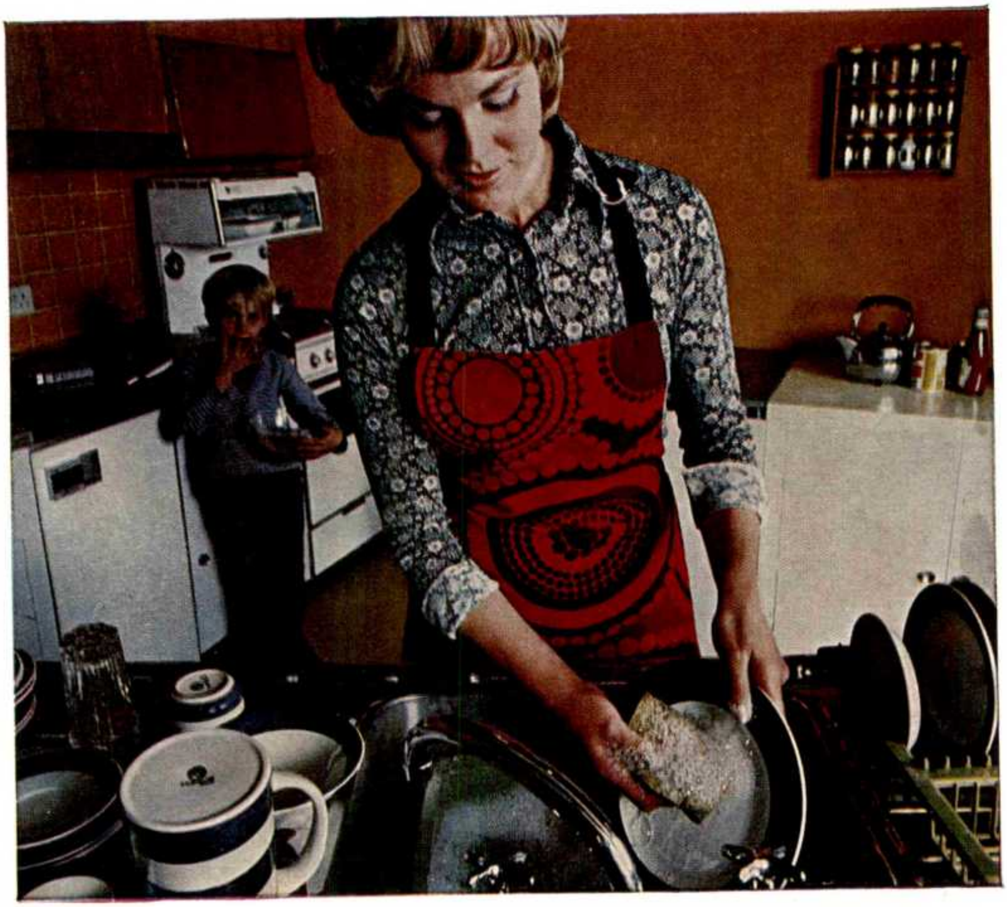

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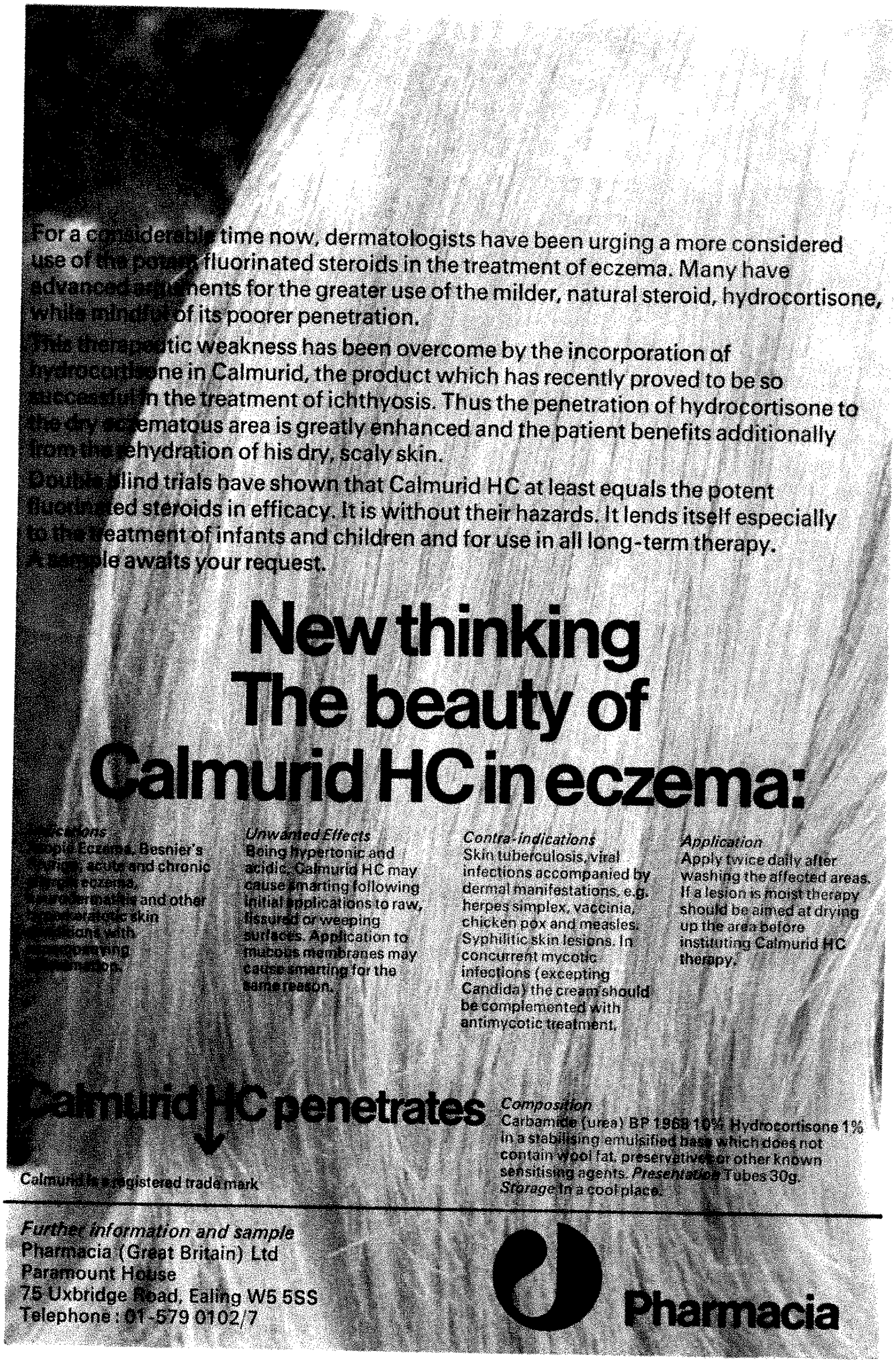

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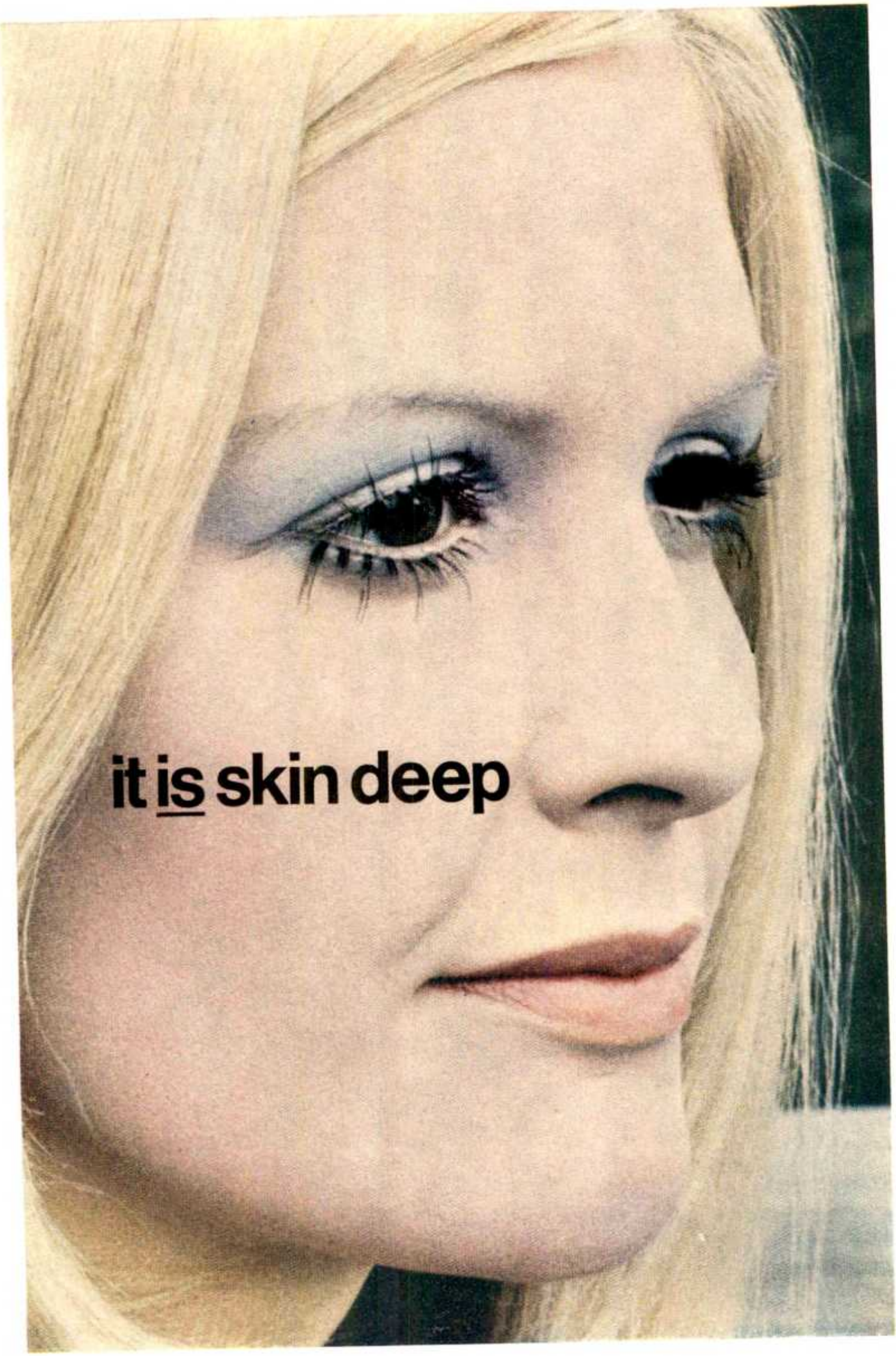
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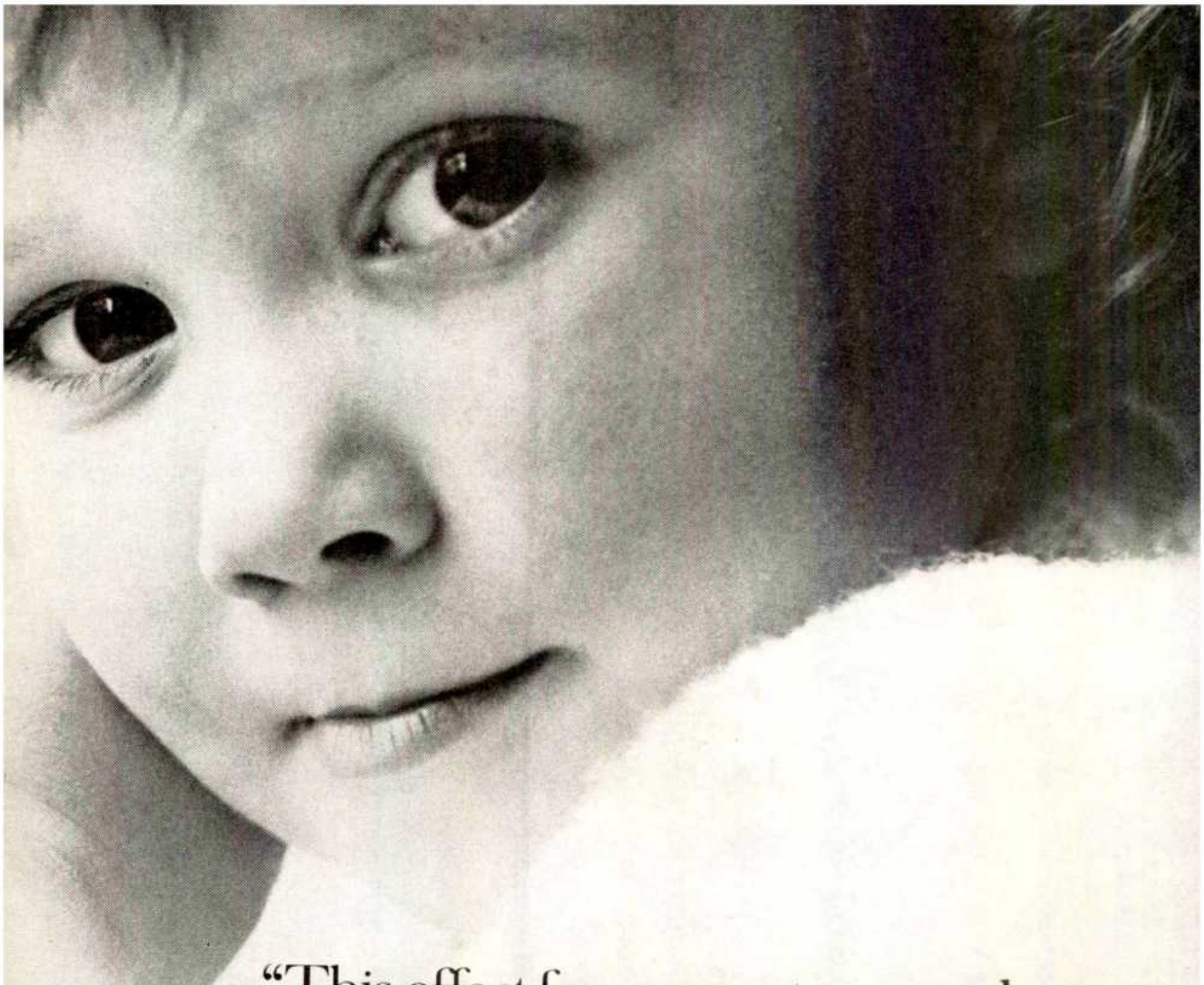

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1. *Clin. Tri. J.*, (1966) *3*: 591
2. *Brit. J. clin. Pract.*, (1967) *21*: 529
3. *Ugeskrift for Laeger*, (1966) *128*: 204
4. *Clin. Tri. J.*, (1966) *3*: 529
5. Paper read to the British Dermatological Association Meeting, Aberdeen, July 1968.

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PITYRIASIS LICHENOIDES: A REAPPRAISAL

R. MARKS, M. BLACK AND E. WILSON JONES

Summary.—One hundred and twenty-eight cases of pityriasis lichenoides presenting at St. John's Hospital for Diseases of the Skin in the past decade have been reviewed. Sixty of these were seen personally, and 44 of these had lesions at the time of interview. Eighteen other patients completed proformata, and adequate hospital records were available for the rest. One hundred and two biopsies from 82 patients with pityriasis lichenoides were also examined in the course of this study. The various clinical manifestations seen in this group of patients has been analysed in order to define the clinical range of the disease. None of the patients studied developed a cutaneous reticulosis and thus the risk for such an occurrence must be small. Patients with pityriasis lichenoides did not appear prone to any systemic complaint, and the disease seemed to be limited to the skin. Some of the patients who had had the disease for several years developed haemorrhagic and necrotic lesions, suggesting that pityriasis lichenoides varioliformis et acuta and pityriasis lichenoides chronica are variants of a single disease process. Although small vessel involvement in the inflammatory process was usual, no histological evidence was obtained to suggest that a vasculitis is of central importance in the pathogenesis of the disease.

When Brocq described the group of conditions that he termed " parapsoriasis " he described one that he called " parapsoriasis en goutes ", which we now know as pityriasis lichenoides (P.L.) (Brocq, 1902). Civatte (1951) subsequently believed that this disorder was not related to the other members of the parapsoriasis group and that it was a basically benign disease. Ingram (1953) studied 32 patients with P.L. and 26 with " parapsoriasis proper ". He also believed that P.L. is a benign disorder. However, there are still some clinicians who believe that a few patients with this disease may eventually develop a cutaneous reticulosis. Samman (1971) has described 8 patients who apparently began with P.L. but later developed poikiloderma and, in some instances, mycosis fungoides. Other similar patients have been shown at clinical meetings (for example, Church, 1970). In addition to this important and unsettled point there is a basic lack of data concerning the natural history of the disease.

For these reasons we have attempted in this study to define the clinical range of pityriasis lichenoides and tried to determine specifically:—

(a) Whether pityriasis lichenoides chronica (Juliusberg) and the acute form (Mucha-Habermann disease) are the same disease;
(b) Whether the disease is limited to the skin;

Paper read at the meeting of the British Association of Dermatology in Edinburgh, July, 1971.
Accepted for publication December 10th 1971.

(c) Whether there is any evidence that the disease can transform into a cutaneous reticulosis;

(d) What role vascular inflammation plays in the pathogenesis of the disorder.

METHODS

The patients that form the basis of this study presented at St. John's Hospital in the decade 1961–71. Cases were traced from the diagnostic index and the histological records of the hospital. Altogether information is available for 128 patients. We have personally interviewed 60 patients, 44 of whom had lesions when seen.

All the patients interviewed were fully examined and were questioned concerning their general health and previous medical history; they were also asked for details concerning their skin disorder. In addition, where possible the following laboratory investigations were performed; skin biopsy, blood picture, liver function tests, serum protein electrophoresis serological tests for syphilis, antinuclear factor test and serological tests for toxoplasmosis. Chest x-rays were also taken when possible.

Proformata were completed by an additional 17 patients, and adequate hospital records were available for the remaining 51. Most of the data presented will concern the 77 patients, either seen personally, or who had completed a proforma. All the cases included in this study were typical in that they had a persistent disorder, characterized by scaly papular lesions, which were on occasions haemorrhagic and necrotic and which tended to come in crops. Where there was doubt, the case was included only if there was good histological evidence for the presence of the disease (Civatte, 1957).

Seven patients who would have satisfied our clinical criteria for inclusion in this study had the histological features of lymphomatoid papulosis and have been reported elsewhere (Black and Wilson Jones, 1971).

One hundred and two biopsies from 82 patients were also available for analysis and especial note was taken of the presence of purpura and vascular damage in these sections.

OBSERVATIONS

Age and Sex Distribution

The age at onset and sex distribution are set out in Table I. It can be seen that it is slightly more common in men than in women in the ratio of 3 : 2 and that it is predominantly a disease of the first half of life (the maximum incidence being in the third decade). However, the age range in the group is wide, as it includes a baby girl age 1 and a man aged 63.

Duration of Disease and Clinical Course Symptoms

The duration of the disorder has varied from a few weeks to more than 20 years at the time of interview. In the 60 patients seen personally the duration of the disease was as seen in Table II.

The rash has lasted more than 20 years in 1 patient. Sixteen patients of this group and 12 of the group who completed proformata were free of rash at the time of writing and the duration of the disease in these 28 was as seen in Table III.

Sixteen of these patients were free of rash in less than 1 year, and it seems that the longer the disease persists the less likely it is to clear spontaneously.

The onset was insidious in most patients, although it was sudden and dramatic in 2. In all patients the disease was markedly fluctuant in character. Either (and most commonly) the disorder was subject to ill-defined periods of relative freedom and bouts of worsening or there were definite attacks of crops and showers of lesions, each attack lasting a few weeks. Some of the patients who appeared completely free remarked that, before finally departing, the number of spots gradually decreased until only the occasional 1 or 2 would appear. Most patients said that they were more prone to exacerbations in the winter and that the exposure to the sun seemed to clear their lesions. In general the disorder was only troublesome cosmetically, although 8 of the 44 patients seen with lesions did admit that it was mildly itchy.

Type and Distribution of Lesions

Forty-four patients had lesions at the time of interview. All had small scaly papules (3–10 mm in diameter). Twenty-six patients had lesions with a circular scale attached centrally and free at the periphery—the typical mica scale (Fig. 1). The lesions of 12 patients showed haemorrhage and/or necrosis (Fig. 2). Six patients had round or oval ulcerated lesions, the craters of which were filled with a thick black eschar (Fig. 3). These ulcerated lesions co-existed with more typical lesions of pityriasis lichenoides. Scars were seen in 9 and depigmented areas in 7 patients (Fig. 4). Four patients had papulosquamous lesions that were "psoriasiform". The lesions of 1 patient (a woman aged 50) had been mistakenly diagnosed as psoriasis when she was first seen. All patients demonstrated lesions at different stages of evolution, and a characteristic feature was the varying depth of redness in different lesions on the same patient. Lesions were distributed on the body as seen in Table IV.

TABLE I.—*Age of Onset and Sex Distribution*

	Males	Females	Totals
0–10	15	11	26
11–20	12	11	23
21–30	20	13	33
31–40	12	6	18
41–50	10	6	16
51–60	4	4	8
61–70	2	2	4
Totals	75	53	128

TABLE II.—*Duration of Rash (at interview)*

3 months	13
3 months–1 year	9
1–2 years	8
2–3 years	6
3–4 years	4
4–5 years	5
5–6 years	3
6–7 years	1
7–10 years	7
> 10 years	4

TABLE III.—*Duration of Rash in "Cured Patients"*

3 months	8
3 months–1 year	8
1–2 years	5
2–3 years	3
3–4 years	1
4–5 years	2
> 5 years	1

TABLE IV.—*Site of Lesions* (44 *patients*)

Arms	40
Legs	36
Trunk	39
Buttocks	26
Face and neck	19
Palms and/or soles	8

The upper limbs were more involved than the lower, and flexor aspects were slightly more frequently affected than the extensor. Often there appeared to be some concentration of lesions around the lower trunk and the buttocks. The lesions were quite dense in some patients, as, for example, in the man aged 50 illustrated in Fig. 5 with histological evidence of P.L.

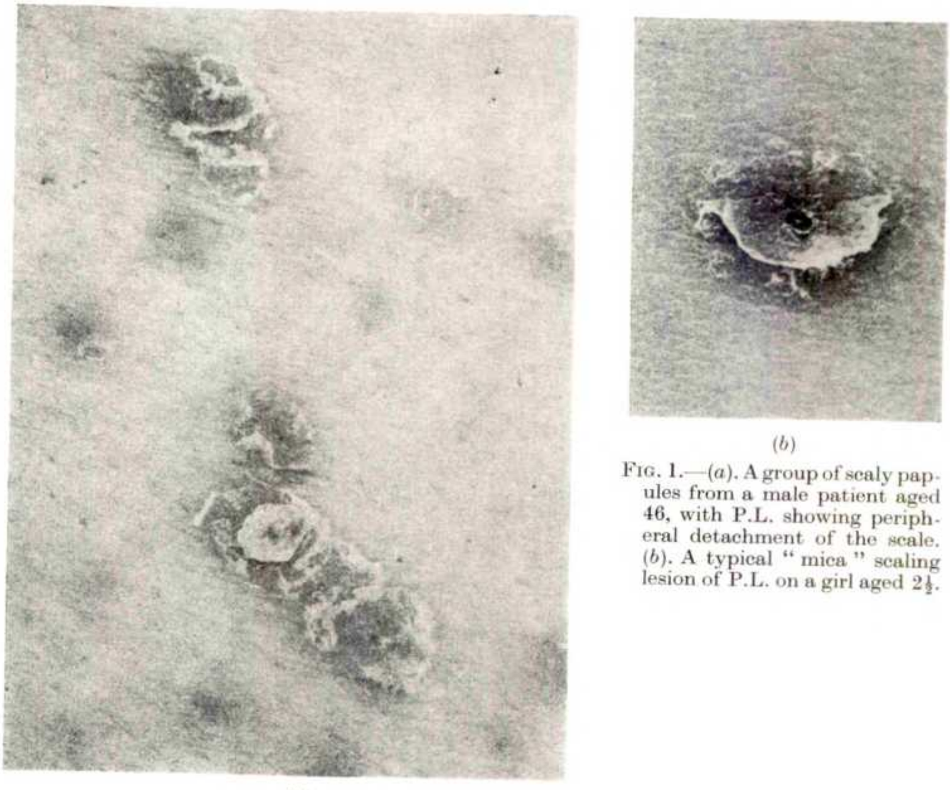
(*a*) (*b*)

FIG. 1.—(*a*). A group of scaly papules from a male patient aged 46, with P.L. showing peripheral detachment of the scale. (*b*). A typical " mica " scaling lesion of P.L. on a girl aged 2½.

" Acute " Lesions

Nineteen patients of the 60 interviewed had lesions that showed necrosis and/or frank bleeding, clinically. The age and sex distribution of these patients and the duration of illness are as seen in Tables V and VI below. The age distribution did not differ markedly from the larger group of patients. Six of these patients have had " acute " lesions that have recurred remittently for more than 5 years.

TABLE V.—*Age (at Onset) and Sex Distribution (Patients with "Acute" Disease)*

	Males	Females
0–10	2	2
11–20	0	4
21–30	3	3
31–40	2	1
41–50	1	1
51–60	0	0
61–70	0	0
Totals	8	11

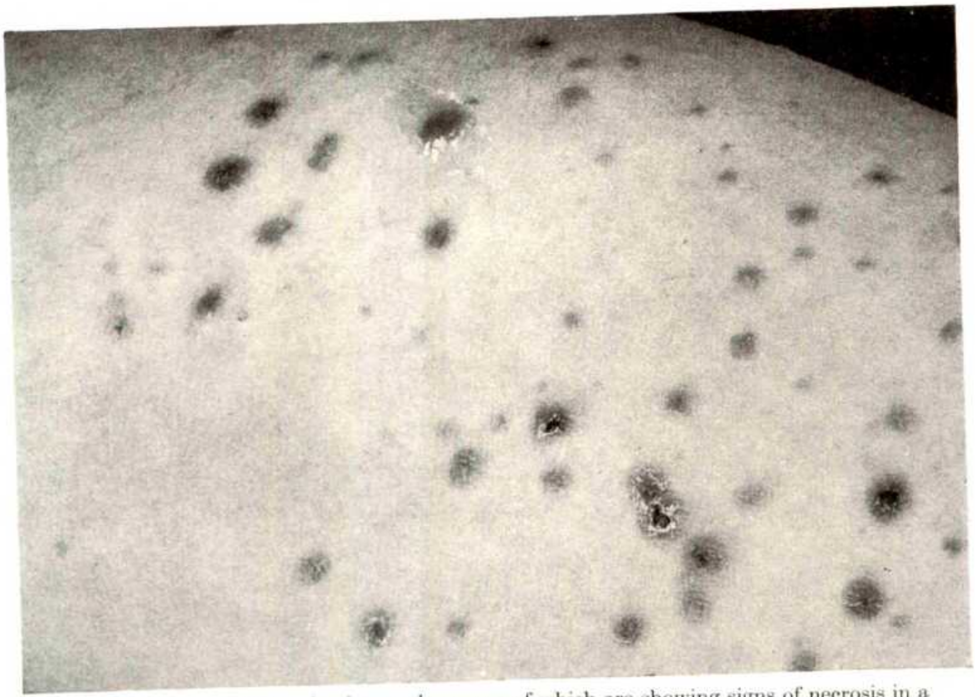

FIG. 2.—Typical haemorrhagic papules, some of which are showing signs of necrosis in a female patient aged 19.

TABLE VI.—*Duration of "Acute" Disease (at Interview)*

3 months	7
3 months–1 year	3
1–5 years	3
5–10 years	4
>10 years	2

Systemic Disease: Coexistence of Lymphoma

One patient, a man aged 30, who had had Hodgkins Disease for 6 years, and who had been treated by radiotherapy, developed the typical scaly papules of pityriasis lichenoides 4·5 years after the onset of reticulosis. No other subject in our series of patients had evidence of a cutaneous or a systematized reticulosis.

No special pattern of systemic disease was observed in this group of patients. One woman, aged 29, with a 15/12 history of P.L., developed systemic lupus erythematosus with severe renal involvement, shortly after the start of her skin disease. At the time of writing she still had both disorders. A man, aged 21, with a 7-year history of P.L., also had regional ileitis. Other complaints noted in this series included peptic ulceration (3), thyroid adenoma (1), diabetes (1) and choroiditis (1). One child, a boy aged 6 from Greece, had thalassaemia minor. No other significant haematological abnormalities were noted in these patients.

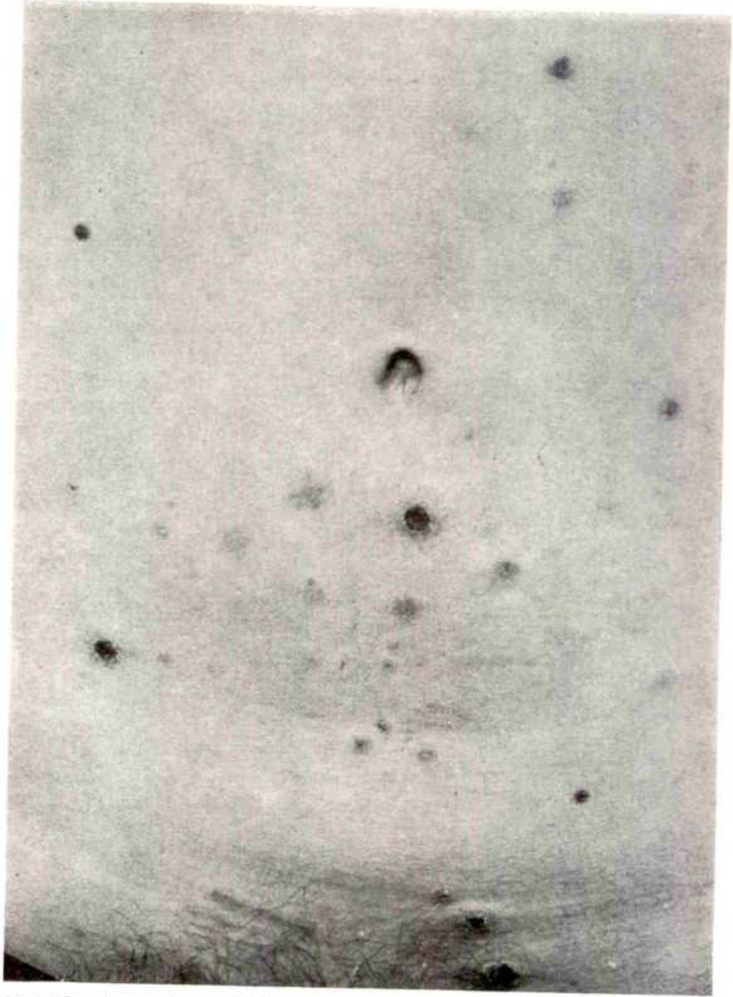

FIG. 3.—Haemorrhagic papules and ulcerated lesions on abdomen of woman aged 23 with P.L.

Serum protein electrophoresis and tests of liver function were performed in 40 patients, and 11 of these showed minor abnormalities in the serum proteins (the most frequent of which was a slight rise in the α-2-globulin). In 74 patients serological tests for syphilis were performed, and these were all negative. Sixteen patients had chest x-ray examinations—all were normal apart from a man of 34 with an 8-year history of P.L. who had an area of fibrosis at 1 apex. Ten patients had negative antinuclear factor tests, 2 patients had positive ANF tests at a titre of 1 : 10; 12 patients had normal titres of antibody to the organism of toxoplasmosis. The 60 patients interviewed had, in addition, no serious abnormalities to general physical examination.

Vasculitis

The histological material available was examined for evidence of vascular involvement. A detailed commentary on the histopathological findings in P.L. forms the basis of a separate report (Marks and Wilson Jones, 1972). In the present investigation special note was taken of vascular involvement in the inflammatory process.

Severe upper dermal oedema was a frequent finding, occurring in 54 of the specimens. Thirty-eight per cent of the 102 biopsies examined showed the presence of free red cells within the dermis. All sections showed a marked perivascular inflammatory cell infiltrate within the upper and papillary dermis. In some sections it appeared that, in addition, some of the deeper dermal blood vessels were surrounded by inflammatory cells. One fifth of biopsies showed a degree of vascular damage, with swelling of the vascular endothelium and even, in a few, fragmentation and eosinophilic change in the vessel wall (Fig. 6). In 3 specimens examined there was undoubtedly a severe degree of vascular damage. However, the sections showing the severe vascular changes described above were those that showed an extreme degree of inflammatory change with frank necrosis and ulceration in some cases. It was therefore difficult to assess whether the vascular damage seen was secondary or was itself responsible for the inflammation.

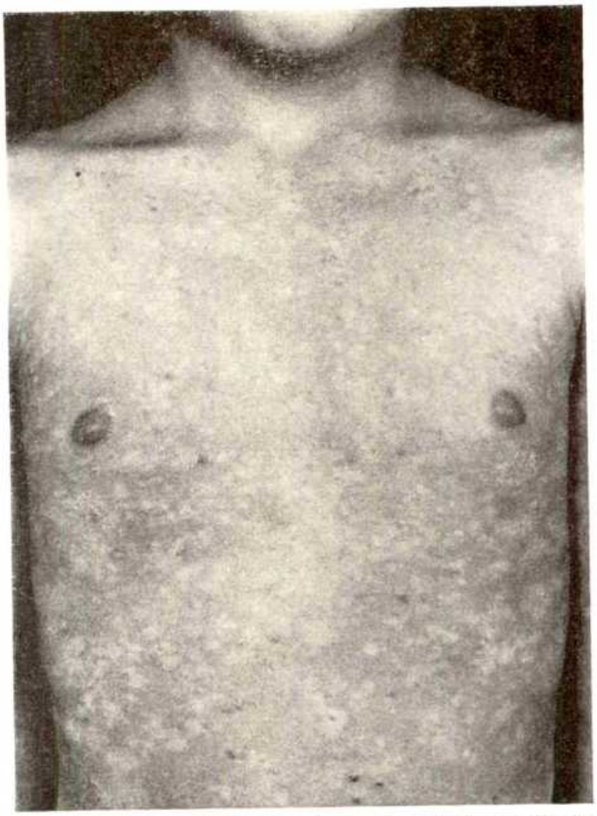

FIG. 4.—Depigmented areas at the site of P.L. papules in a boy aged 6 with a 2-year history of the disease.

DISCUSSION

Berardi *et al.* (1967) have extensively reviewed the literature concerning the acute type of P.L., as well as describing 8 cases of their own. The reader is referred to this article for a very full historical account of the subject. Ingram (1953) described his experience with 32 patients with P.L. and with 26 who had " parapsoriasis proper ". He believed that P.L. never transformed into a reticulosis and was a benign, self-limiting disorder, easily separable from " parapsoriasis ". Certainly, there no longer seems to be any justification for the lumping together of P.L. with other persistent scaly disorders which are difficult to classify. However, the historical fact of its prior inclusion in the parapsoriasis group seems to have influenced thought as to its aetiology and its relationship to the reticuloses. Indeed, we believe that the term " parapsoriasis " has outlived its usefulness and would be better discarded. To retain it gives credence to the view that there exists a specific type of chronic disorder which transforms into mycosis fungoides. In fact, it is likely that mycosis fungoides develops *ab initio*, and we agree with Montgomery (1968) that it is usually possible to detect its presence histologically throughout the disease, even in the early " parapsoriatic stage " of the disorder

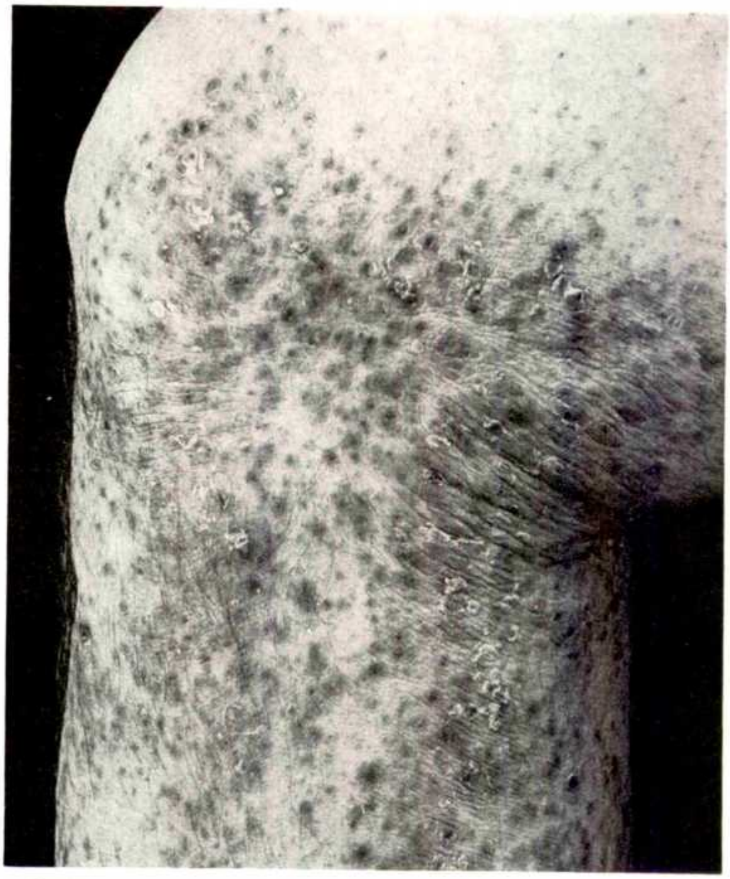

FIG. 5.—Clinical photograph from a man aged 50 with P.L., illustrating that the rash can be profuse.

Relationship to Cutaneous Reticuloses

We believe that, in many cases where there seems to have been the development of a reticulosis in the course of P.L., either (a) the initial diagnosis was incorrect and the pityriasis-like papules seen were those associated with a prereticulotic poikiloderma, or (b) the case is one of lymphomatoid papulosis.

The relationship between P.L. and lymphomatoid papulosis and the possibility of this latter complaint becoming truly lymphomatous have been discussed at length by Black and Wilson Jones (1971). Lymphomatoid papulosis is almost certainly a variety of P.L. in which there are many unusually large and bizarre mononuclear cells. Two patients with this or an allied disorder seem to have developed a true lymphoma subsequently and died. The first was Case 1 of Kawada *et al.* (1969) and the other a case of Black and Wilson Jones (1971). Approximately 7% of biopsies from patients with P.L. have a small number of large and abnormal-appearing monocytoid cells (Marks and Wilson Jones, 1972), and it seems that the difference between lymphomatoid papulosis and P.L. is only a matter of degree. As a corollary of this it would seem reasonable to suppose that there is an increased likelihood of patients with P.L. developing a reticulosis. It was, therefore, unexpectedly that we found that there was no evidence for progression

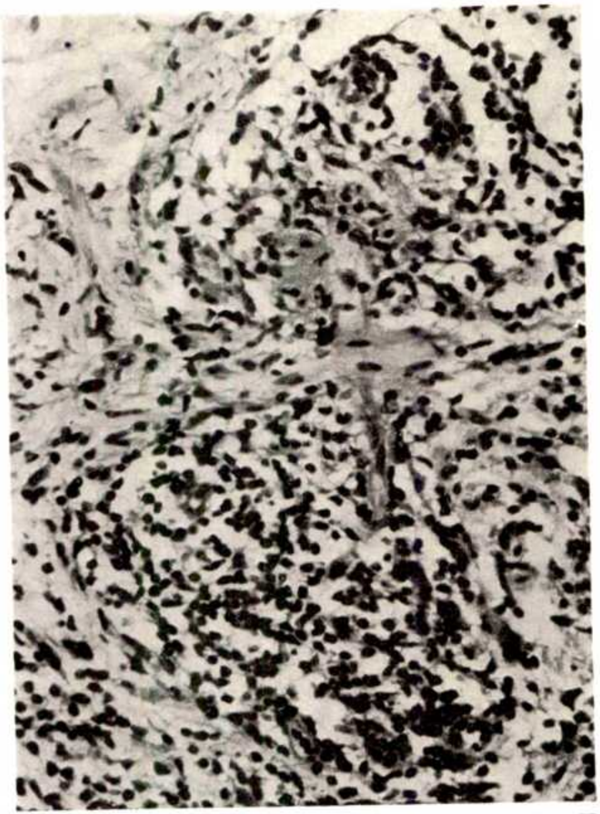

FIG. 6.—Photomicrograph to show vascular damage in a lesion of P.L. H. and E. × 180.

of P.L. to a reticulosis in any of the 128 patients that we reviewed. It does appear, however, that the scaly papules seen in a prereticulotic poikiloderma may lead to confusion with P.L., as may the misinterpretation of biopsies from patients with P.L. containing the large bizarre cells mentioned above. A further source of difficulty is that severe scarring, resulting from ulcerated lesions of P.L., and pigmentary incontinence can simulate the appearance of poikiloderma. We know of one patient, a young man, where this was indeed the case. We do not wish to imply that there is no association between P.L. and reticulosis, only that there are pitfalls in the diagnosis of the same condition in both patients. It appears that, if patients with P.L. are at risk as far as the development of lymphoma is concerned, the risk must be very small.

It should be emphasized that patients were admitted to this series only if there was no doubt concerning their diagnosis. If there were signs of poikiloderma, or other evidence of a cutaneous reticulosis co-existing with a P.L.-like disease at initial presentation, the case was not then included. To have included such patients would not have answered the question as to whether P.L. can transform into a reticulosis.

Involvement of Blood Vessels

Szymanski (1959) introduced the concept of a vasculitis to explain the haemorrhage and necrosis in the lesions of pityriasis lichenoides varioliformis et acuta. He examined 22 biopsies from 18 patients with the " acute " disease and 37 biopsies from patients with the chronic type of disease. Szymanski was impressed with the perivascular lymphocytic infiltrate, the invasion of the vessel walls, the endothelial cell swelling and the local haemorrhage in biopsies of the acute type of lesion. He believed that the changes were those of a vasculitis. He described his findings in the chronic type of P.L. as " astonishingly slight ", and believed that this disorder is basically a disease of the epidermis. We have presented clinical evidence above to suggest that pityriasis lichenoides varioliformis acuta and pityriasis lichenoides chronica are merely variants of a single disease, and similarly that biopsies of " acute " lesions and " chronic " lesions show similar pathological changes but with shifts of emphasis (Marks and Wilson Jones, 1972). In both types of lesion a perivascular lympho-histiocytic infiltrate is usual. In the more florid types of lesion there is also upper dermal oedema, and a variable amount of bleeding and endothelial cell swelling, but these changes may also be seen to a lesser extent in less inflamed lesions. Definite fibrinoid necrosis, leukocytoclastic angiitis and other evidence of vasculitis were not found in our material (although 3 patients with the ulcerative type of lesions did show pronounced vascular involvement with slight eosinophilia of the vessel walls and blurring of the vascular margins). It would be fair to say that, while the small dermal vessels seem to be "caught up" in the inflammation, there is no evidence from our studies that vascular involvement plays an initiating, perpetuating or central role in the pathogenesis of the disease.

" *The Acute Disease* "

Degos *et al.* (1966) described 2 patients with very severe ulcerative pityriasis lichenoides who were persistently pyrexial in the course of their illness. Burke *et al.* (1969) described 2 similar patients and termed this very severe type of

P.L. " Febrile Ulceronecrotic Mucha Habermann's disease ". None of the patients in our series was as severely affected as those described by Degos or Burke and their colleagues, but several of our patients had a number of ulcerated and necrotic lesions similar to those described by these authors. We have been impressed by the appearance of these " acute type " lesions in patients who have had P.L. for several years. We have, in addition, looked after patients with typical "chronic" scaly papules where the disease has remitted (apparently permanently) after a few months. Furthermore, most patients with P.L. have lesions which exhibit differing shades of dark red as if there were a petechial element to some of them. From Table V it can be seen that the age structure of patients with " acute lesions " is similar to that of all the patients with P.L. In our view, and from a consideration of the above, it does not seem justified to separate " acute " and " chronic " types of disease purely on a morphological basis, as acute and chronic lesions seem to represent extreme ends of the same spectrum.

Is P.L. Limited to the Skin?

In general, patients with P.L. did not seem to differ from the general population as far as their experience of systemic disease was concerned. However, we were surprised to find that 11 of the 40 patients tested showed minor abnormalities in the serum protein electrophoretic strip. The signficance of this finding is unknown and is being further investigated.

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JUVENILE DERMATITIS HERPETIFORMIS IN JAMAICA: CLINICAL AND GASTROINTESTINAL FEATURES

JOYCE WARNER, S. E. H. BROOKS, W. P. T. JAMES AND SHEILAH LOUISY

Summary.—The clinical features and investigations relating to gastrointestinal function are presented in 12 cases of juvenile dermatitis herpetiformis seen over a period of 2 years in Jamaica.

None of the children had evidence of steatorrhoea. Jejunal biopsy in 10 cases showed normal mucosa in 1, ridging and convolutions in 8 and a flat mucosa in 1. Electronmicroscopical changes are also described. Lactase deficiency and lactose intolerance were common. These findings may be related to malnutrition in infancy or at the time of biopsy.

An interesting feature was relapse following iodine contrast media used during jejunal biopsy and a high incidence of iodine in drinking water in some parts of Jamaica.

A DEFINITE relationship between disease of the skin and of the intestine has recently been found in adult dermatitis herpetiformis (Marks *et al.*, 1966; van Tongeren *et al.*, 1967; Fraser *et al.*, 1967; Fry *et al.*, 1967; Marks *et al.*, 1968; Bendl and Williams, 1968; van der Staak *et al.*, 1970). Approximately one-half to two-thirds of these patients had a jejunal mucosa closely resembling that seen in coeliac disease. Three children have recently been described with coeliac disease and juvenile dermatitis herpetiformis (Mayon-White, 1969; Meenan and Cahalane, 1969; McNeish *et al.*, 1970).

In Jamaica we have found a surprising predominance of juvenile dermatitis herpetiformis rather than the adult form of the disease. Over a period of 2 years we saw 12 patients under the age of 11 years, and we present the clinical features and our findings relating to gastrointestinal function and structure.

MATERIALS AND METHODS

Faecal fat excretion. This was measured by the method of van der Kamer (1949). Patients were on an ordinary ward diet, and faeces were collected over 3 days.

Xylose tolerance test. 0·4 g xylose,0·9 g/kg body weight, was given, and blood samples for xylose were taken before the loading dose, and then after 1 h and 2 h.

Lactose tolerance test (McMichael *et al.*, 1965). After an over-night fast, 2 g/kg body weight of lactose were given orally. Total reducing substances in the blood were measured before giving lactose and then at half-hourly intervals.

Serum iron and total iron binding capacity (TIBC). These were measured by the method of Beale *et al.* (1962).

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Serum folate. This was measured by the method of Spray (1964).

Serum calcium, alkaline phosphatase and total proteins were estimated with an autoanalyser by the methods of Williams and Moser (1953), Powell and Smith (1954) and the biuret reaction, respectively.

Plasma inorganic phosphate was measured by Gomori's technique (1942) and glucose-6-phosphate dehydrogenase by the rapid screening method of Sass *et al.* (1966).

Skin biopsies were available from 11 patients. The specimens were formalin-fixed and, after routine processing, sections were stained with HE.

Jejunal biopsies were obtained by the modified technique of James (1968), using a paediatric Watson capsule. The tissue was divided into 3 for enzyme assays, morphological and histological studies and electron microscopy.

(*a*) *Enzyme assays.*—The mucosal sample was immediately weighed and then frozen with dry ice, disaccharidase activity being estimated within 24 h of biopsy. Lactase, sucrase, palatinase and maltase activities were measured on 1–5 mg of mucosal tissue by Burgess *et al.* modification (1964) of Dahlqvist's method (1964). Results were expressed in terms of the wet weight of mucosal tissue.

TABLE I.—*Clinical Details*

Case no.	Age (years)	Sex	Percentile		Duration of disease	Main site of blisters	Response to dapsone	Spontaneous remission in hospital
			Weight	Height				
1	10	M	25th	75th	2 years	Trunk, limbs	Good	—
2	?5	F	*	*	29 months	Hands, feet, neck	None	Yes
3	2½	F	50th	90th	21 months	Lower legs	Good	Yes
4	3½	F	75th	50th	20 months	Limbs, trunk	Good	—
5	4	M	25th	Below 10th	1 year	Genitalia, legs	Good	—
6	7½	M	10th	25th	1 month	Genitalia, thighs	Fair	No
7	7½	F	70th	50th	5 years	Legs	Fair	Yes
8	?5	M	*	*	2 years	Trunk, limbs	Good	—
9	4	F	90th	90th	2½ years	Legs, arms	Fair	Yes
10	2	F	80th	90th	1 month	Generalized	None	No
11	5½	F	—	—	4 months	Limbs	Good	Yes
12	5	M	20th	30th	1 year	Lower legs and arms, head	Good	Yes

* Percentiles cannot be given, as the exact age of the children is unknown.

(*b*) *Morphological and histological studies.*—Part of the jejunal biopsy was orientated by placing the non-villous surface on a filter paper. It was fixed in 4% formaldehyde, examined under a dissecting microscope and photographed. After careful positioning, sections were cut and stained by standard histological procedures. The morphology of the biopsies was graded numerically as described by Shuster *et al.* (1968): Grade 1—villous; Grade 2—ridged; Grade 3—convoluted; Grade 4—flat. Surface epithelium measurements of the sectioned mucosa were made with a micrometer eyepiece, with 20 estimations for each biopsy. Villous and crypt heights were also measured.

(*c*) *Electronmicroscopy.*—Small (0·5 mm) blocks of mucosa were fixed immediately for electronmicroscopy in 2·5% phosphate-buffered glutaraldehyde, pH 7·4 at 4°C. After 2 h the blocks were rinsed overnight in 0·2 mol/l buffer and post-fixation was carried out for 1 h in 1% phosphate-buffered osmium tetroxide. After dehydration through graded alcohols, the blocks were embedded in Maraglas. Sections (1·0 μ) were cut for orientation, and surface areas selected for study. Thin sections were cut with glass knives on an LKB Ultrotome, picked up on copper grids, and stained with uranyl acetate and lead citrate. Electronmicroscopy was carried out with a Philips 100C at 60 kV.

CLINICAL INFORMATION

None of the children had a family history of skin disease and none had a story of fatty stools or diarrhoea. Eleven of the children were of Negro descent and 1 was East Indian (Table I). Some case histories are given below.

Case 2.—She had many non-itchy blisters up to 3 cm in size, chiefly on her feet, hands and neck. In hospital she was given dapsone* for 2 weeks, but her blisters failed to respond. She became jaundiced with a high reticulocyte count. Sulphapyridine was substituted, but she developed crystalluria and severe haematuria. Finally, prednisone 45 mg daily was given, with no response. She was sent home without drug treatment and, for over a year, was never free from blisters for longer than a week. When re-admitted to hospital for jejunal biopsy, she had a spontaneous remission lasting 3 weeks, but she relapsed severely after a week at home.

Case 10.—She was the most severe case in this series. Her trunk, limbs, face and head were all involved. In particular she had blisters on her eyelids, tongue and lips, both the vermilion and the labial mucous membrane. She did not respond to dapsone 100 mg daily, and her reticulocyte count rose to 9%. Sulphapyridine 1·5 g daily was therefore substituted and she became almost free from lesions. A jejunal biopsy was done, during which 8 ml of uromiron (250 mg inorganic iodine per ml) was given, and she had a severe temporary relapse within 24 h. At present, she is free from blisters and being maintained on sulphapyridine.

TABLE II.—*Haematological Findings*

Case no.	Haemo-globin (g/100 ml)	White blood count (per mm³)	Eosino-phils (per ml blood)	Peripheral blood film	Serum iron (μg/100 ml) (normal 50–120)	TIBC (μg/100 ml) (normal 250–350)	Glucose-6-phos-phate dehydro-genase
1	12·1	5400	216	Normal	71	296	Normal
2	11·4	7000	350	Hypochromic	92	284	Normal
3	10·7	8100	972	Hypochromic	85	370	Absent
4	11·0	9600	384	Normal	97	322	Normal
5	11·5	5400	486	Hypochromic	48	288	Normal
6	12·0	13,200	1056	Normal	60	288	Normal
7	11·6	8000	560	Normal	121	378	Normal
8	10·4	9100	3000	Anisocytosis	78	395	Normal
9	11·0	5900	885	Hypochromic	52	482	Normal
10	9·9	6900	340	Hypochromic (on oral iron)	—	—	Normal
11	11·1	13,600	680	Hypochromic	—	—	—
12	10·0	13,000	4416	Hypochromic	71	385	Normal

LABORATORY FINDINGS

Four of the children were on or below the 25th percentile in weight compared to normal Jamaican schoolchildren (Ashcroft *et al.*, 1965; Ashcroft and Lovell, 1966) (Table I). The results of haematological investigations are shown in Table II. Eight children had an eosinophilia of over 440 eosinophils per ml; in 3 of these there was associated intestinal infestation with worms. Blood films were hypochromic in 7 cases and the saturation of plasma with iron was below 20% in 3. The serum folate was below 2·5 mg/ml in 3 cases. Despite the iron deficiency, only 1 case had a haemoglobin below 10 g/100 ml.

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* Dapsone—Diaminodiphenylsulphone.

Serum calcium and phosphate, alkaline phosphatase and serum proteins were normal in all cases.

The results of tests done to investigate intestinal function are shown in Table III. Faecal fat excretions were all normal. Six children had xylose tolerance tests, and 1 was abnormal. Seven children had lactose tolerance tests: 5 had a rise in plasma-reducing substances of less than 25 mg, only 2 being within normal limits.

TABLE III.—*Gastrointestinal Findings*

Case no.	Faecal fat (g/day)	Xylose (mg/100 ml) 1 h	Xylose (mg/100 ml) 2 h	Rise in plasma-reducing substances after lactose (mg/100 ml)	Serum folate (mμg/100 ml)	Intestinal parasites
Normal values	< 5	< 25		< 25	< 2·5	
1	0·6	48	45	—	4·1	None
2	1·4	22	31	—	6·3	None
3	1·1	23	25	19	2·1	*Ascaris lumbricoides* and *Giardia lambia*
4	1·8	16	23	—	4·0	None
5	0·33	48	48	—	5·15	Threadworms
6	1·0	50	50	20	2·6	None
7	0·66	—	—	4	9·2	None
8	—	—	—	15	2·35	*Ascaris lumbricoides*
9	0·6	—	—	22	1·15	*Trichuris trichuria*
10	0·6	—	—	70	2·6	None
12	0·7	—	—	38	5·45	*Ascaris lumbricoides*

TABLE IV.—*Disaccharide Activity in Jejunal Mucosa*

(μmol/min/g wet weight of tissue)

Case no.	Lactase	Sucrase	Palatinase	Maltase
Normal values	> 1	> 5	> 1	> 15
1	0·44	11·0	2·07	72·0
2	3·44	12·2	2·40	91·5
3	4·63	19·0	2·08	48·6
4	1·90	10·8	1·11	38·5
5	0	5·7	2·10	67·6
6	0·79	12·8	1·19	44·0
7	1·77	23·2	5·27	71·2
8	0	4·3	1·08	8·1
9	—	—	—	—
10	0·21	5·7	0·22	15·0
11	—	—	—	—
12	0	5·7	2·07	15·1

The disaccharide activities of the jejunal mucosa in 10 children are shown in Table IV. Four children had normal enzymes. The rest had low or absent lactase, 2 of these associated with other enzyme deficiencies.

Histology

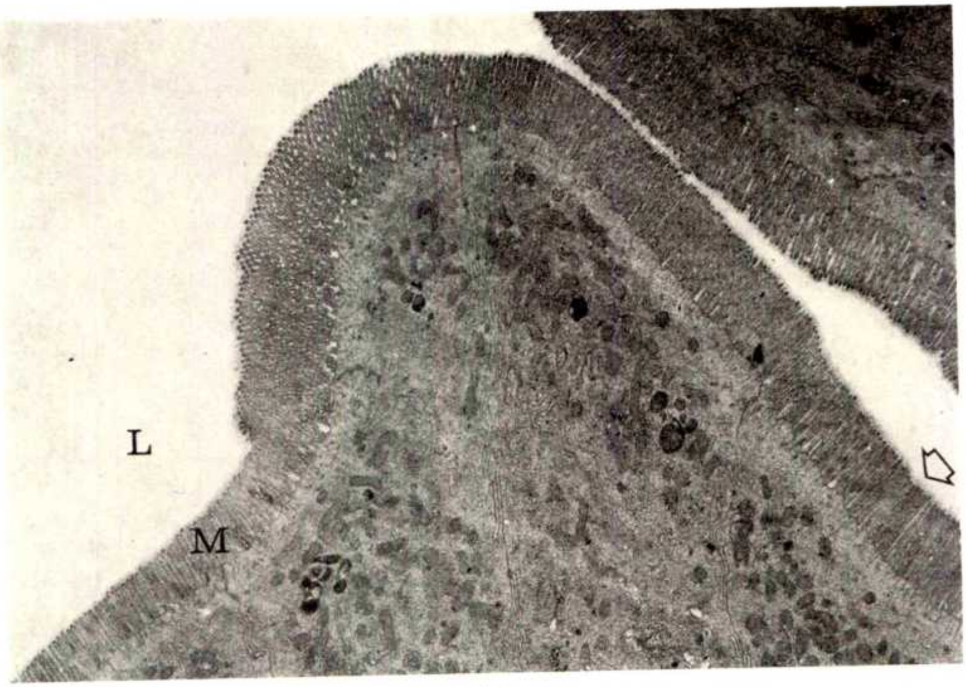
In 11 cases skin biopsies were available; 10 showed a sub-epidermal blister. In and around the blister was a cellular infiltrate containing eosinophils. The

TABLE V.—*Jejunal Mucosal Biopsy Appearances*

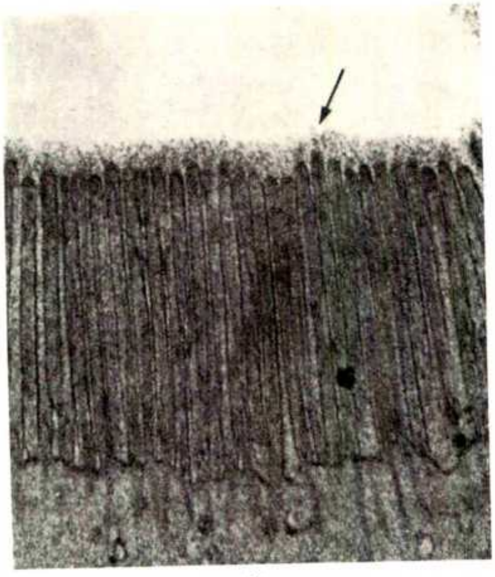
Case no.	Stereo-microscopy	Cellular infiltration	Villous height (mean, range μm)	Crypt height (mean, range μm)	Surface cell height (mean ± SD range μm)	Grade	Electronmicroscopic alterations		
							None	Mild	Marked
1	Ridged	++	244 (104–325)	246 (169–325)	28 ± 4 (18–40)	2			+
2	Convoluted	+	356 (325–390)	Difficult to assess	29 ± 4 (15–36)	3		+	
3	Convoluted	++	183 (80–190)	213 (100–310)	27 ± 4 (20–32)	3	+		
4	Ridged	++	208 (63–300)	170 (138–200)	22 ± 10 (11–38)	3	Not done		
5	Convoluted	++	178 (0–286)	581 (260–728)	24 ± 9 (9–35)	3	+		
6	Ridged	++	287 (60–380)	Difficult to assess	33 ± 8 (15–48)	2	+		
7	Villous	+	376 (200–475)	158 (87–250)	33 ± 6 (14–44)	1	+		
8	(Specimen too small for histological study)								
9	Ridged	++	185 (100–250)	240 (163–375)	31 ± 12 (24–38)	2		+	
10	Flat	+++	Flat	Difficult to assess	20 ± 4 (15–30)	4			+
12	Convoluted	++	244 (79–490)	207 (165–275)	24 ± 6 (18–31)	3	+		

remaining biopsy showed an intraepidermal blister, probably due to biopsy of a late lesion.

Jejunal histology was examined in 10 patients, and the pathological findings



(*a*)



(*b*)

FIG. 1.—(a) Electronmicrograph of jejunal mucosa (Case 5), showing normal appearances with regular, evenly-shaped microvilli (M) on the luminal surface (L) (× 10,000).

(b) Higher magnification of area from Fig. 1(a), showing normal uniform microvilli, and coating of mucopolysaccharide on luminal surface (arrow) (× 30,000).

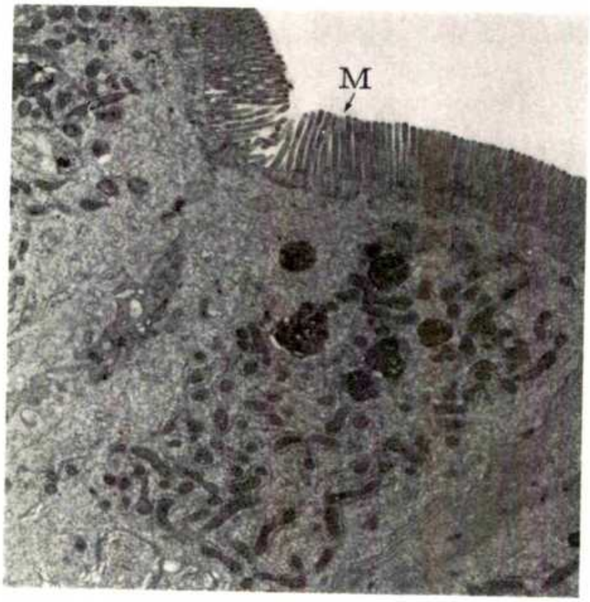
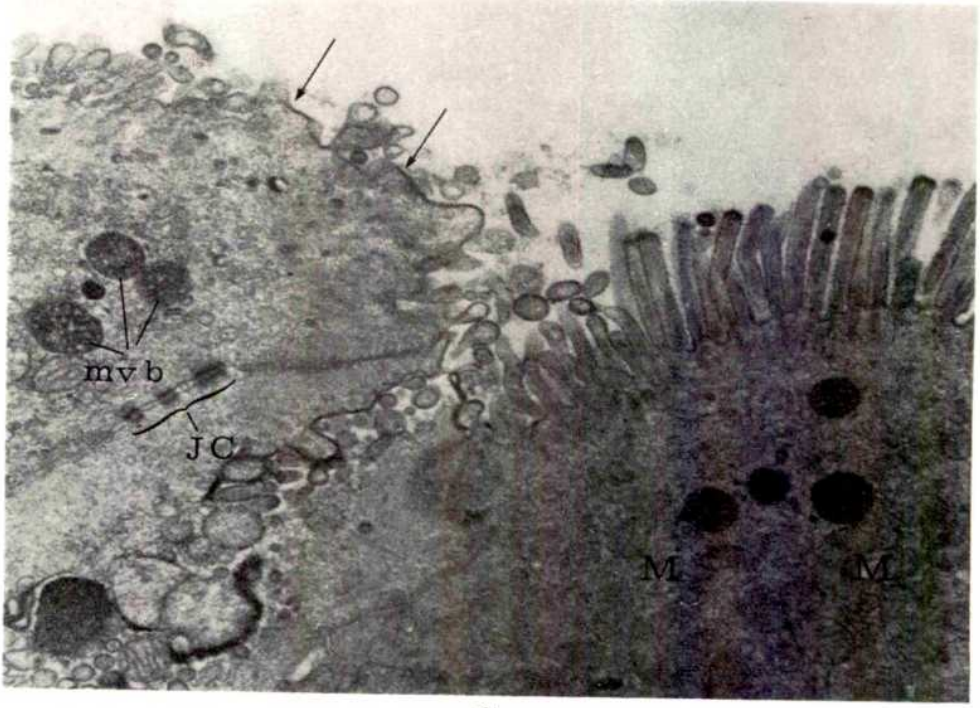


FIG. 2.—Electronmicrographs of 2 areas from jejunal biopsy (Case 9). (a) In this area the microvilli (M) appear normal, except that no surface coating of mucopolysaccharide is seen (×11,800).
(b) An epithelial cell showing marked blunting and irregularity of the microvilli (arrows) (M—mitochondria; J.C.—junctional complex; mvb—multivesicular bodies) (×32,400).

(*a*)



(*b*)

are shown in Table V. The classification of different mucosal types is based on the predominant lesion seen. There was a normal villous pattern in only 1 patient; 8 showed ridging or convolutions; 1 had a flat mucosa. The child with the flat mucosa was Case 10 with the most marked skin abnormalities. Reduction of villous height and increase of crypt height were also seen in the majority of cases, together with a reduced epithelial cell height.

Electronmicroscopy of the jejunal biopsies was done in 9 cases. In 5, the fine structure of the surface cells appeared normal, as illustrated in Fig. 1a, b. In 2 others there were focal alterations consisting of shortening and irregularity of the microvilli (Fig. 2b). The remaining 2 biopsies showed epithelial cell degeneration with vesiculation of the endoplasmic reticulum, loss of the lateral plasma membrane infoldings and irregularities of the microvilli (Fig. 4a, b)., such as occur in coeliac disease (Biempica *et al.*, 1968).

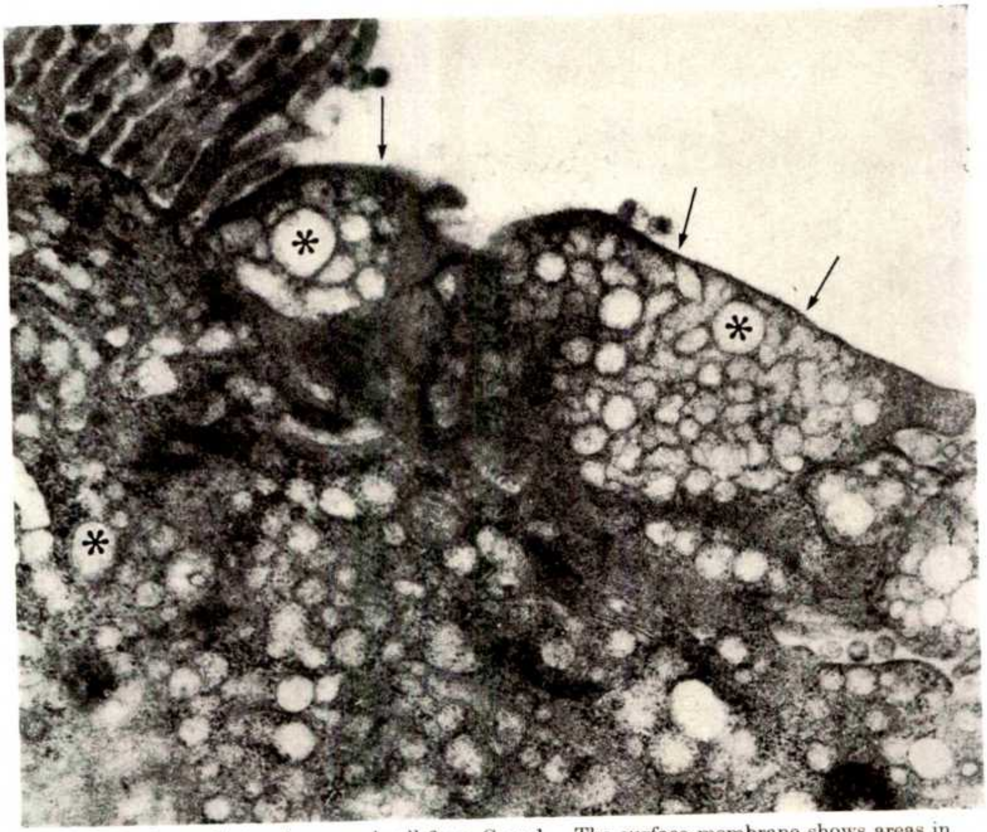


FIG. 3.—Surface of jejunal mucosal cell from Case 1. The surface membrane shows areas in which microvilli are absent (arrows), and there is vesiculation of the endoplasmic reticulum (*). The fibrous zone is not visible (× 32,400).

DISCUSSION

The skin disease in our patients does not resemble typical dermatitis herpetiformis as seen in adults, but these differences have been previously noted by

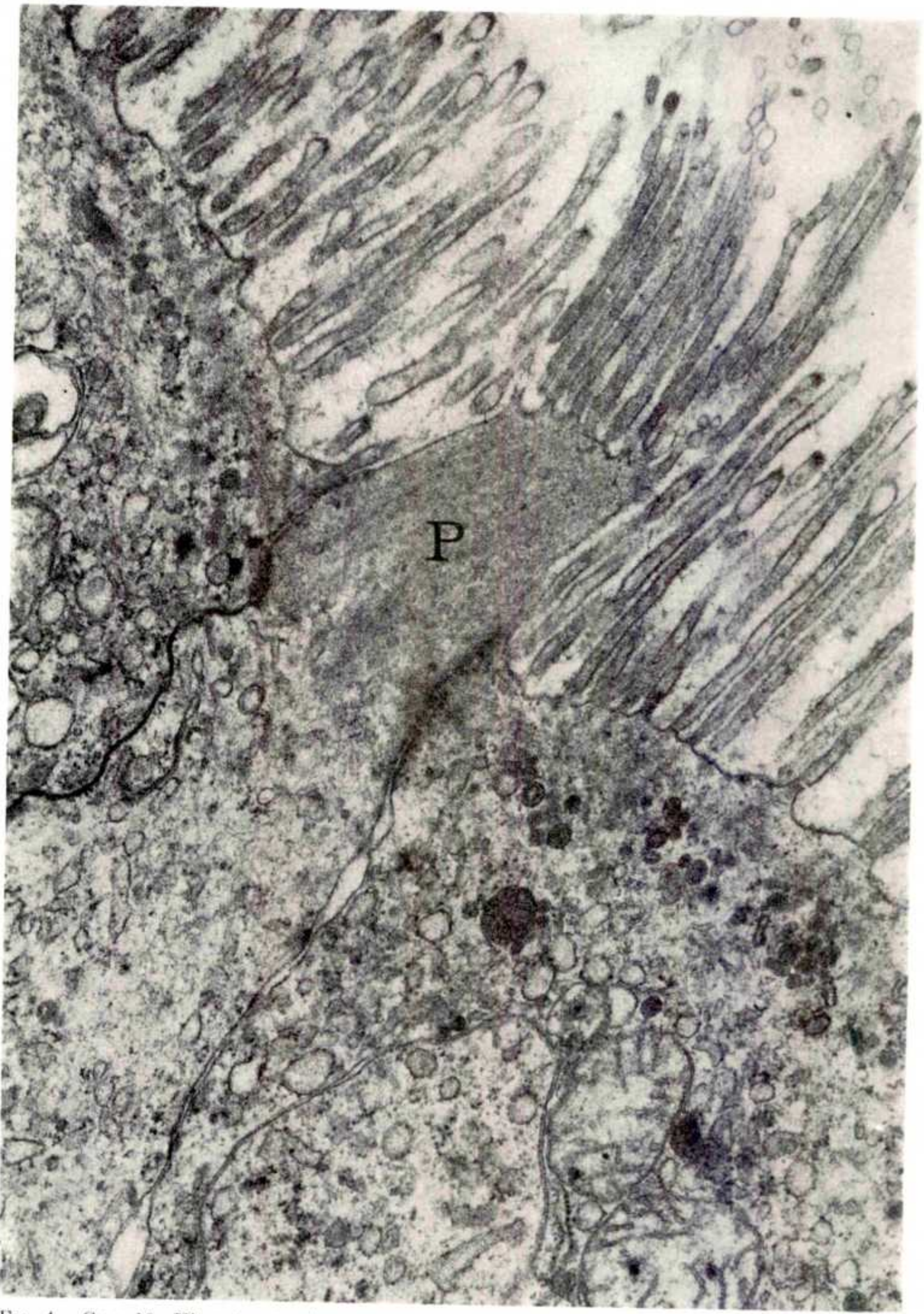


FIG. 4.—Case 10: Ultrastructural appearance of 2 areas of the luminal border of jejunum: (a) The microvilli are irregularily spaced. The middle cell shows a cytoplasmic protusion (P) (×32,400); (b) sparse microvilli in clumps (*) (×30,800).

several authors, including Gardiner (1909), Knowles (1907), Sobye (1946), Berlin (1952), Tolman *et al.* (1959), Kim and Winkelmann (1961), Ganpule (1967) and Grant (1968). We feel that our cases were typical of what is called the juvenile form of dermatitis herpetiformis, or juvenile pemphigoid. The blisters varied in size from about 2 mm to 4 cm, and many were intact, irritation being an infrequent feature. The distribution of the lesions was asymmetrical and predominantly on the limbs, with frequent involvement of genitalia, thighs, scalp and neck. Only 1 patient had mucous membrane involvement. Healed lesions tended to leave temporarily depigmented areas (Fig. 5), but no permanent scarring. Seven cases responded well to dapsone, 3 had some improvement, and a further case had a definite but less satisfactory response to sulphapyridine. Only 1 child failed to respond to drug therapy. Spontaneous remissions were much more common than in adults, and this was particularly noticeable when the children were admitted to hospital. For this reason and the fact that gluten-free flour is not available in Jamaica, we were unable to assess the effect of a gluten-free diet. Meenan (1969) found in his case of juvenile pemphigoid with coeliac disease that the skin underwent spontaneous remission although a diet was not adhered to, and McNeish (1970) reported a child whose dermatitis herpetiformis developed while his coeliac disease was in remission.

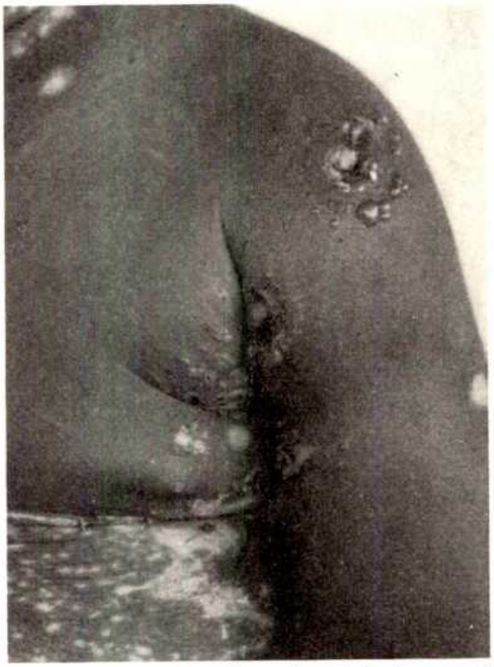


FIG. 5.—Case 10: Blisters on left arm and trunk with depigmentation from old lesions.

None of our patients had evidence of steatorrhoea or generalized malabsorption. Mild anaemia was common, and intestinal parasites were frequently found, but there appeared to be no correlation between the severity of jejunal mucosal changes and worm infestation.

It is known that, in the Caribbean Islands, the morphology of the jejunal mucosa may vary from that generally accepted as normal in Caucasoids (Ten Thije, 1968). In Jamaican children malnutrition produces both lactase deficiency and mucosal abnormalities which return towards normal when malnutrition is corrected (Sparke and James, 1968; James, 1970), but no jejunal biopsies have been performed on normal Jamaican children.

Marks and Shuster (1970) found that 8% of the population in Newcastle on Tyne had jejunal mucosal abnormalities, and they felt that this was explicable on the basis of variation in the normal mucosa between one geographical region and another. It was notable that all our cases came from a low socio-economic stratum, and 4 had mild degrees of malnutrition, so this may be the explanation of the mucosal abnormalities that we found. The lactase deficiency and lactose intolerance may represent the results of malnutrition in infancy, as lactase is particularly sensitive to mucosal damage and recovers more slowly than the other disaccharides (Sheehy *et al.*, 1966).

It seems remarkable that juvenile dermatitis herpetiformis should be so common in Jamaica. A similar situation seems to exist in Scotland (Grant, 1968). Over the past 2 years we have seen only one case in an adult. This is in contrast to findings at the Mayo Clinic where, of 1150 cases of dermatitis herpetiformis, only 49 began before the age of 15 years, an incidence of 4·3% (Kim and Winkelmann, 1961). We wondered about the relationship to local factors, especially diet. In 4 of our cases relapse was related to iodine given as a contrast medium in the course of jejunal biopsy. The deleterious effect of iodine was first noted by Besnier and Doyon in 1891 (Kaposi, 1891). Sea fish is one of the staple diets in Jamaica, and most of the children are dosed regularly with cod-liver oil in some form, which contains approximately 0·015 mg iodine per ml. In addition, in some areas of the island, the water supply contains remarkably large amounts of iodine, varying from 3 parts per billion of inorganic iodine at the University Hospital to 320 parts per billion in a specific rural area. Sea water contains 10 parts of iodine per billion (Ellington, 1969). It would be interesting to know what part, if any, iodine may play in the frequency of the disease in Jamaica and whether the spontaneous remissions in hospital were related to the lower iodine content of the water.

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CHRONIC MUCOCUTANEOUS CANDIDIASIS, ENDOCRINE DEFICIENCY AND ALOPECIA AREATA

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Summary.—A family with the endocrine candidiasis syndrome is described, in which 3 members with idiopathic hypoparathyroidism also suffered from oral and cutaneous candidiasis. Alopecia areata developed in 1 of the affected members, A review of 42 cases described in the literature revealed 13 patients with alopecia areata. The eyelashes or eyebrows were involved in 12 patients. Addison's disease appears to be commoner in those patients with the syndrome who also have alopecia areata. The evidence from this retrospective analysis is not conclusive and merits further observation, but these findings are in keeping with a possible autoimmune aetiology for alopecia areata.

THORPE and Handley (1929) were the first to describe the association between idiopathic hypoparathyroidism and candidiasis. This syndrome has now been shown to include idiopathic adrenal insufficiency (Leonard, 1946; Craig and Schiff, 1955; Whitaker *et al.*, 1956), Hashimoto's disease (Kenny and Holliday, 1964; Carter *et al.*, 1959), pernicious anaemia (Morse *et al.*, 1961; Hung *et al.*, 1963; Strickland, 1969), chronic active hepatitis (Kunin *et al.*, 1963), kerato-conjunctivitis (Gass, 1962) and steatorrhoea (Morse *et al.*, 1961). The cause of the disease is not known. However, the familial (Sutphin *et al.*, 1943; Drury *et al.*, 1970) and hereditary (Sutphin *et al.*, 1943) nature of the disease is accepted. The finding of various organ-specific antibodies in the patients (Blizzard *et al.*, 1967; Irvine *et al.*, 1967), and their relatives (Wuepper and Fudenberg, 1967), the characteristic histological changes seen in the affected organs (Gass, 1962) and the spectrum of cell-mediated immunological abnormalities (Block *et al.*, 1971) favour an autoimmune mechanism. Lehner (1966) reviewed 37 patients, suggested the useful term "endocrine candidosis syndrome" and reported 6 patients with alopecia. Blizzard *et al.* (1967) reported 3 patients with alopecia totalis in 16 patients with Addison's disease, all of whom were suffering from idiopathic hypoparathyroidism. Irvine *et al.* (1967) found no difference in the incidence of alopecia in patients with idiopathic as compared with patients with tuberculous adrenal insufficiency. The presence of alopecia areata in 1 of the patients to be described prompted a review of the hair changes reported in previous cases.

CASE REPORTS

Case 1.—*Hazel C.* was born in 1951 and, at the age of 3, developed a condition resembling malabsorption syndrome, which settled spontaneously after 1 year. Hepatomegaly and splenomegaly were noted at this time, and, at the age of 5, a liver biopsy showed features "compatible with the late effects of hepatitis". At the age of 13

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she was investigated for failure to thrive. D-Xylose absorption was impaired, but a jejunal biopsy was not performed. Tetany, associated with low serum calcium levels, was attributed to malabsorption, and she was started on a gluten-free diet with vitamin supplements.

At 15 she was admitted to a medical ward in Aberdeen. Examination revealed a girl of small stature, with poorly developed breasts and absent axillary and scanty pubic hair. She had a few spider naevi and several dystrophic nails (Fig. 1). The blood pressure 95/70. Serum calcium 3·6 mEq/l, serum inorganic phosphate 3·7 mEq/l, S.G.O.T. 108 units, S.G.P.T. 99 units, total plasma proteins 9·8 g/100 ml, γ-globulin 3·33 g/100 ml. Plasma levels of IgG, IgA and IgM were normal. Barium meal and follow-through examination revealed multiple jejunal diverticula and a skull x-ray showed slight calcification of the basal ganglia. Hypoparathyroidism was diagnosed and treatment was started with ergocalciferol and calcium supplements.

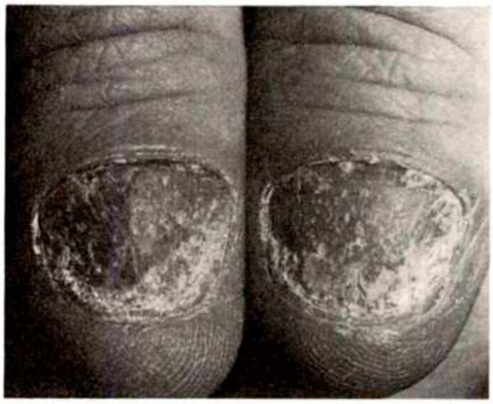
FIG. 1.—Changes in finger nails in case 1.

Bilateral perceptive deafness was diagnosed at the age of 16 and ophthalmological examination at the age of 17 revealed bilateral subcapsular cataracts and bilateral cystic degeneration of the macula. At about the same time she was started on prednisone therapy for her liver disease, which was then diagnosed as chronic active hepatitis. A severe respiratory infection at the age of 18 led to a further hospital admission and bronchiectasis was confirmed. Examination again showed underdeveloped breasts and scanty pubic hair, with erythema and scaling of the palms and interdigital webs, dystrophic nails, angular stomatitis and very red oral mucosa. Candida was cultured from nail clippings and palm scrapings. Anti-thyroid anti-cytoplasmic antibody was strongly positive, and the thyroglobulin tanned cell test gave a titre of 1 in $2{\cdot}5 \times 10^6$. She has no goitre and has remained clinically and biochemically euthyroid. The anti-mitochondrial antibody test was positive, but anti-nuclear, anti-gastric and anti-smooth muscle antibody tests were negative.

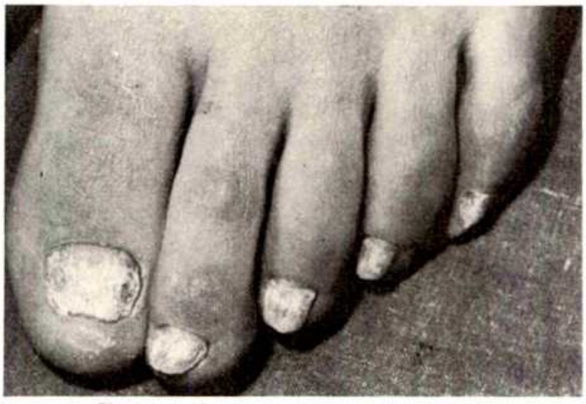
FIG. 2.—Changes in toe nails in Case 2.

Hazel's present therapy consists of prednisone 5 mg daily, ergocalciferol 50,000 units on alternate days and 1 Sandocal tablet daily. Maintenance of normal serum calcium levels and treatment with anti-candidal agents have had no beneficial effect on her candidiasis.

Case 2.—*Kathleen C.*, the non-identical twin of Hazel, has had deformed finger and toe nails since the age of 1 year. At the age of 17 she was examined in the dermatology department and was found to have, in addition to the dystrophic nails (Fig. 2), redness and scaling of both palms, interdigital webs and both soles. Both eyebrows and eyelashes were sparse and the hairs easily pulled out. There were exclamation mark hairs. The oral mucosa was red and she had angular stomatitis. Scrapings of the palms and nail clippings grew candida on culture, but cultures of oral swabs were negative. One year later Kathleen developed occasional facial twitching and carpal spasm. These symptoms became more troublesome and she was investigated in Aberdeen Royal Infirmary in 1969. Serum calcium levels were 3·9, 3·8, 3·9, 4·0 and 3·5 mEq/l and the corresponding serum inorganic phosphate levels were 3·3, 3·1, 2·9, 3·2 and 4·5 mEq/l. Total serum proteins 7·6 g/100 ml, γ-globulin 1·5 g/100 ml. Plasma levels of IgG, IgA and IgM were normal. Tests of thyroid, adrenal and liver function were normal. Tests of antibodies to thyroid gland, parietal cells, intrinsic factor, mitochondria and smooth muscle were all negative, and the anti-nuclear factor was negative. Treatment with ergocalciferol and calcium was started and she is now taking a maintenance dose of 75,000 units of ergocalciferol and 3 Sandocal tablets daily. There has been no improvement in the condition of Kathleen's nails or hair while her calcium levels have been normal.

Case 3.—*David C.*, brother of Hazel and Kathleen, was born in 1955. Since the age of 8 he had complained of chronic infection and deformity of his finger nails; at the same time he developed fissuring at the angles of his mouth, and an oral infection which cleared with Nystatin lozenges. He was investigated in Aberdeen Royal Infirmary at the age of 14, after complaining of carpal spasm and twitching of his eyelids while brushing his hair. Serum calcium levels were 4·2, 4·3, 3·9, 3·4 and 4·0 mEq/l and the serum inorganic phosphate levels were 3·9, 4·3, 4·4, 3·5 and 4·3 mEq/l. Total serum proteins 7·8 g/100 ml, γ-globulin 1·3 g/100 ml. Plasma levels of IgG, IgA and IgM were normal. Tests of adrenal, thyroid and liver function were normal. Nail culture grew *Candida albicans*, and degenerate yeasts were seen on a film of mouth scrapings. Antibody tests to thyroid, parietal cells, intrinsic factor, smooth muscle and antimitochondria were all negative. His serum calcium level is now satisfactorily controlled by ergocalciferol 50,000 units daily and Sandocal, 2 tablets daily. He is also taking 3 Nystatin lozenges each day.

Neil C., brother of Hazel, Kathleen and David, was born in 1961 and has no complaints referrable to his skin, nails or hair. He was fully investigated in 1970 and shown to have normal parathyroid, thyroid, adrenal and liver function. Tests of antibodies to thyroid gland, parietal cells, mitochondria and smooth muscle were negative, and anti-nuclear antibody was negative.

Helen C., born in 1925 is the mother of the 4 children described above and is free of skin and endocrine disease. However, she became prematurely grey at the age of 20 years, and has a strongly positive anti-cytoplasmic anti-thyroid antibody. Antibody tests to parietal cells, mitochondria and smooth muscle were negative, and the anti-nuclear antibody was negative. Helen C. has one sister with pernicious anaemia, and another sister whose daughter complained of an episode of paraesthesia in both hands, and twitching of her eye which settled spontaneously. Another niece is known to have diabetes insipidus.

Norman C. was unrelated to Helen C. before their marriage and has no personal or family history of skin or endocrine disease.

REVIEW OF THE LITERATURE

For the purposes of this review a patient was included only if the hair status was mentioned in the case report. Of the 42 cases reported in the literature there are 27 cases in which hair changes are described, and these have been listed in Table I. In Table II the site and type of hair change is listed and related to systemic disease. In Table III the type and site of hair change is related to the frequency of organ involvement.

The scalp hair was affected in 24 of the 27 patients described (89%). A diagnosis

TABLE I.—*List of Hair Changes Described in the Literature*

Carter *et al.* (1959)	Progressive loss of scalp hair. Scalp and eyebrows sparse and fine and easily pulled out
Craig and Schiff (1955)	
Case 2	Patchy areas of alopecia aged 7 persisting for 5 years until death. Post-mortem histology; hair follicles lacking in hair shafts and their epithelium thin vacuolated and atrophic. Diffuse inflammatory infiltrate around hair follicles. No fungal elements.
Case 3	Circumscribed areas of alopecia with raised erythematous edges aged 7. Alopecia spread to involve eyebrows. Scalp lesion resistant to antifungal therapy. Regrowth of hair aged 11
Drury *et al.* (1970)	
Case 1	Patchy alopecia with depilation eyebrows and eyelashes aged 5. Complete alopecia aged 8. Regrowth aged 9. Recurred aged 10. Alopecia totalis aged 15. Post-mortem; body completely devoid of hair aged 24.
Case 2	Patchy alopecia scalp aged 11
Emerson *et al.* (1941)	
Case 1	Scalp hair thin and patchy and eyebrows and eyelashes sparse aged 15. Scalp hair thin and patchy. Axillary and pubic hair absent and eyelashes and eyebrows scanty aged 25
Fields *et al.* (1971)	Diffuse non-scarring alopecia of scalp. Remaining hair coarse and dry and thinning of eyebrows and pubic hair aged 15.
Forbes (1956)	
Case 2	Scalp hair brittle and grew no longer than 0·5 in–1·0 in aged 3. Severe hair loss after starting calcium and parathormone—striking loss of hair from eyebrows and scalp aged 3½. Still bald after 3 years treatment with AT10 and calcium
Case 3	Brittleness and partial loss of hair aged 3. Hair became finer after vitamin D and calcium aged 11
Case 5	Some loss of hair aged 10
Case 7	Hair began to fall out easily aged 3. Total alopecia after vitamin D and calcium aged 5. One year later, whilst still on therapy, almost total regrowth of hair which was dry and brittle.
Gass (1962)	
Case 2	Alopecia aged 5. Partial alopecia aged 15
Hermans *et al.* (1969)	
Case 2	Hair loss of alopecia areata type present for 8 years and examination showed alopecia of right temple, and scanty eyebrows, axillary and pubic hair aged 29
Case 3	Lock of hair at front of scalp became white aged 7. Eyelashes scanty, white and short, and few small areas of alopecia of the scalp noted aged 27. Episode of alopecia areata aged 32
Kenny and Holliday (1964)	
Case 1	Patchy alopecia and no axillary and pubic hair aged 15.
Kunin *et al.* (1963)	Sparse beard, axillary and pubic hair aged 16. Hair fine and streaked with grey and scanty axillary and pubic hair aged 20
Leonard (1964)	Alopecia aged 9½. Scalp hair then fine and silky and patches of baldness and absent pubic hair aged 10. Sparse hair aged 12
McMahon *et al.* (1959)	Patient lost much of hair aged 7. Hair grey-streaked and sparse axillary and pubic hair aged 21
Morse *et al.* (1961)	
Case 1	Scalp hair thin aged 18
Case 2	Scalp hair fine aged 11
Papadatos and Klein (1954)	Alopecia areata: large confluent areas of baldness of scalp. A few hairs comprised eyelashes and eyebrows aged 5½. No response to treatment for hypoparathyroidism
Sutphin *et al.* (1943)	
Case 3	Scalp hair thin and sparse and eyebrows absent aged 12
Thorpe and Handley (1929)	Scalp hair dry but not coarse
Whitaker *et al.* (1956)	Hair of legs, arms, eyebrows, axillae and genitals sparse and scalp hair dry and sparse aged 15. Few months later area of alopecia left side of scalp
Wuepper and Fudenberg (1967)	Scalp hair normal. Axillary and pubic hair sparse aged 20

of alopecia areata was considered likely in 13 (44%). The eyebrows were affected in 10 patients (37%), axillary hair in 7 (25%), pubic hair in 9 (33%) and eyelashes in 4 (15%). The parathyroids were affected in 25 patients (93%), adrenal cortex

TABLE II.—*Site and Type of Hair Change Related to Systemic Disease*

Author	Case no.	Hair status					Systemic disease
		Scalp	eyelashes	eyebrows	axilla	pubis	
Carter *et al.* (1959)		+		+			P.A.T.
Craig and Schiff (1955)	(2)	A.A.					P.A.
	(3)	+		+			P.A.
Drury *et al.* (1970)	(1)	A.A.	+	+			P.A.T.G.
	(2)	A.A.					P.A.
Emerson *et al.* (1941)	(1)	A.A.	+	+	+	+	P.
Fields *et al.* (1971)		+		+		+	P. + vitiligo
Forbes (1956)	(2)	+		+			P.
	(3)	+					P.A.
	(5)	+					P.
	(7)	+					P.
Gass (1962)	(1)	N					P.A.
	(2)	A.A.					P.G.
Hermans *et al.* (1969)	(2)	A.A.		+	+	+	A.
	(3)	A.A.	+				P. + vitiligo
Kenny and Holliday (1964)	(1)	A.A.			+	+	P.A.T.
	(2)	N					P.
Kunin *et al.* (1963)		A.A.			+	+	P.A.
Leonard (1946)		A.A.				+	P.A.
McMahon *et al.* (1959)		A.A.			+	+	P.A. + vitiligo
Morse *et al.* (1961)	(1)	+					P.G.
	(2)	+					P.A.G.
Papadatos and Klein (1954)		A.A.	+	+			P.A.
Sutphin *et al.* (1943)	(3)	+		+			P.
Thorpe and Handley (1929)		+		+			P.
Whitaker *et al.* (1956)		A.A.		+	+	+	P.A.
Wuepper and Fudenberg (1967)		N			+	+	A.T.
Total 27	24 (89%)	A.A.13 (44%)	4 (15%)	10 (37%)	7 (25%)	9 (33%)	

+ = Affected.
A.A. = Alopecia areata.
N. = Normal.

P—Hypoparathyroidism (25).
A—Addison's (16).
T—Hypothyroidism (4).
G—Pernicious anaemia (4).
Vitiligo (3).

TABLE III.—*Type and Site of Hair Change Related to Frequency of Organ Involvement*

Sites affected	No.	Systemic disease			
		P	A	T	G
All patients	27	25 (93)	16 (59)	4 (15)	4 (15)
Scalp—alopecia areata	13	12 (92)	10 (77)	2 (15)	2 (15)
Scalp—normal and non-alopecia areata	14	13 (93)	6 (43)	2 (14)	2 (14)
Eyelashes	4	4	2	1	1
Eyebrows	10	9	6	2	1
Axilla	7	5	6	2	0
Pubis	9	7	7	2	0

P—Idiopathic hypoparathyroidism.
A—Addison's disease.
T—Hypothyroidism.
G—Pernicious anaemia.
Percentage of total in parentheses.

in 16 (59%), thyroid gland in 4 (15%) and gastric parietal cells in 4 (15%). Adrenocortical hypofunction occurred in 6 patients (43%) without alopecia areata, but was present in 10 patients (77%) with alopecia areata. This difference, however, is not significant (Fisher's exact test). Vitiligo was described in 3 patients (11%).

DISCUSSION

The affected patients in the family described were considered to be suffering from the "endocrine candidosis syndrome". Two had hair changes, 1 in the form of alopecia areata. The cause of alopecia areata is not known and, although there is a significant association between alopecia areata and thyroid disease, including Hashimoto's thyroiditis (Muller and Winkelmann, 1963; Cunliffe *et al.*, 1969), there does not appear to be a significant association with the presence of thyroglobulin antibodies (Cunliffe *et al.*, 1969).

This was a retrospective review. However, even allowing for a number of misdiagnoses, there does appear to be a high incidence of alopecia areata in patients with the endocrine candidiasis syndrome. Alopecia of the scalp could result from organ dysfunction, but it is unlikely in patients in whom the hair changes preceded the systemic upset (Papadatos and Klein, 1954; Forbes, 1956, Cases 2 and 7; Drury *et al.*, 1970, Case 1; Hermans *et al.*, 1969, Case 2; Gass, 1962, Case 2), and in those in whom treatment did not improve the hair condition (Forbes, 1956).

Although figures are not available for the frequency of involvement of the eyelashes and eyebrows in alopecia areata, it is sufficiently uncommon to merit a report of 4 cases seen over a 2-year period (Kile, 1960). It is not possible from the descriptions in this review (Table I) to be certain whether these patients had true alopecia areata of the eyelashes and eyebrows. The hair changes could be secondary to organ dysfunction, but this is very unlikely in Case 2 (Kathleen C.) described above, and in 2 others (Papadatos and Klein, 1954; Drury *et al.*, 1970), since the hair changes preceded the onset of endocrine dysfunction. In Forbes's case (1956), and Case 2 (Kathleen C.) hypoparathyroidism was the only associated systemic disease, and its treatment had no effect on the hair condition. These observations suggest that patients with alopecia areata, who have involvement of eyelashes or eyebrows, should be screened for endocrine candidiasis syndrome. Hair changes could result from an infective process to which these patients are particularly susceptible, but attempts to demonstrate organisms have been unsuccessful (Craig and Schiff, 1955). The histological changes not uncommonly seen in the skin in alopecia areata are reminiscent of the changes in the thyroid gland in Hashimoto's thyroiditis. These include accumulation of lymphocytes, atrophy of neighbouring structures such as hair follicles and sebaceous glands, and subsequent fibrosis (McLeod, 1920). Craig and Schiff (1955) reported similar histological changes in the scalp of a patient with the endocrine candidiasis syndrome.

The other feature of interest in this survey is the greater frequency of Addison's disease in those patients with the endocrine candidiasis syndrome who have, in addition, alopecia areata. This finding requires confirmation. The criteria which have been used to establish an autoimmune basis for Addison's disease (*Lancet*, 1969) include the presence of antibodies against the adrenal cortex and, often, other organs, and the clinical association with, and histological similarities to, other autoimmune diseases. A similar argument can be made for alopecia areata. The histological appearances have been described above and the strong clinical associations stressed. It has not been possible to show a significant association with the presence of thyroid autoantibodies (Cunliffe *et al.*, 1969). However, in this group of patients, the parathyroids and adrenal cortex were involved more frequently than the thyroid gland, and it may be more rewarding to search for autoantibodies to the former organs. Clearly, the detection of anti-hair antibodies will strengthen the argument in favour of an autoimmune basis for alopecia areata.

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DEMONSTRATION OF BASAL CELL IMMUNOFLUORESCENCE IN DRUG-INDUCED TOXIC EPIDERMAL NECROLYSIS

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Summary.—In 2 patients with toxic epidermal necrolysis due to penicillin and chlorpromazine respectively, direct immunofluorescent studies revealed intercellular fixation of immunoglobulins and complement globulins confined to the basal cell layer of the epidermis. These findings suggest that the drug binds to intercellular epidermal protein and that the basal cells are the target site of cellular damage in drug-induced epidermal necrolysis.

WHILE the clinical features and course of toxic epidermal necrolysis (TEN) are well recognized, the pathogenesis remains unclear. In infancy and childhood infection with Staphylococcus phage 71 seems to be of etiological importance, and a necrolytic factor may be operative (Samuels, 1967; Arbuthnott *et al.*, 1971). These patients usually recover without *sequelae.* Drugs have been suspected in adult cases, the mortality of which approaches 25% (Lyell, 1967). Phenylbutazone, phenolphthalein, procaine and sulphonamide have been major causative agents (Koblenzer, 1967). Other drugs recorded are oil of chenopodium, aloin, am-barbital sodium, tetrachloroethylene, penicillin, tetracycline, Mesantoin, actinomycin, gold salts, promethazine, aspirin, phenobarbitone, phenacetin and Meperidine (Potter *et al.*, 1960; Lowney *et al.*, 1967).

Histologically, reports have varied as to the exact site of cleavage and include clefts occurring at all levels of the malpighian layer, in addition to subepidermal bulla formation (Koblenzer, 1967; Walker, 1962). Characteristically cleavage occurs in the absence of dermal inflammation.

Electron microscopic findings in TEN suggest that separation of the epidermis from the dermis occurs at the dermo-epidermal junction. The desmosomes and the basement membrane are intact morphologically (Komura *et al.*, 1969).

Specific immunofluorescent findings have been well documented in pemphigus and bullous pemphigoid. This communication extends the usefulness of immunofluorescence to the study of drug-induced TEN.

CASE REPORTS

Case 1.—A girl aged 1 year was admitted to the hospital of the University of Pennsylvania with a severe blistering skin eruption. Her health had been excellent

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until 6 days before admission, when she developed a high fever with a cough, for which she received an intramuscular injection of procaine penicillin. Within 2 days she became afebrile and asymptomatic. Ten days later she again became ill, with a high fever and an earache. Otitis media was diagnosed and a penicillin suspension was prescribed. Four days after initiation of penicillin therapy, an erythematous macular eruption developed on her face. This spread progressively to involve her trunk, arms and legs. Penicillin was stopped, but she remained toxic, and her skin disease continued to progress.

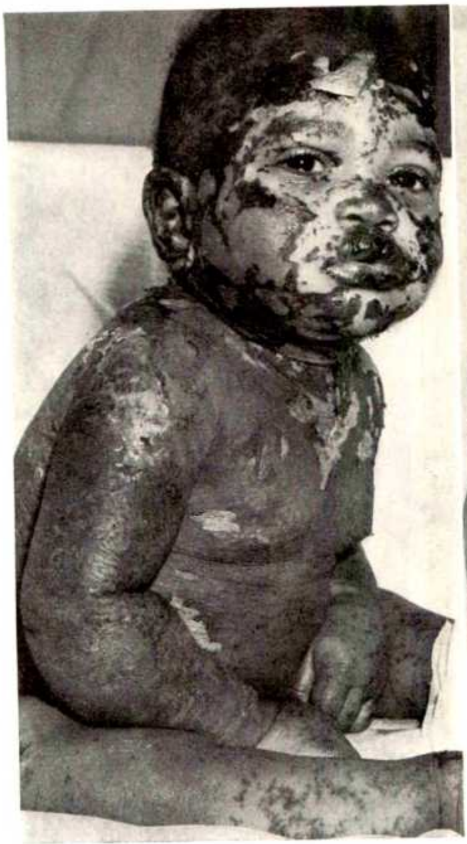

FIG. 1.—Denuded erythematous areas present on the face, neck and trunk of a baby aged 1 year with TEN. Purpuric lesions involved the extremities.

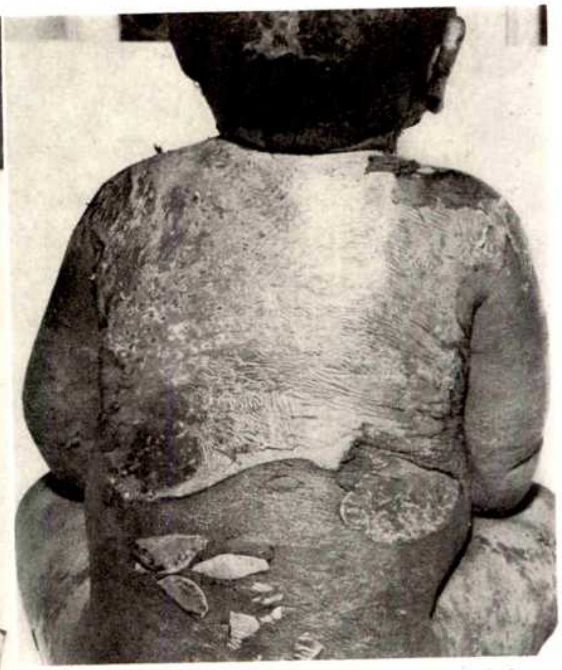

FIG. 2.—The back of the baby shows loss of the epidermis in large sheets. The Nikolsky sign was positive.

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On examination, the patient appeared acutely ill and obviously in pain with the slightest movement; temperature 41·5°C, respirations 36/min, pulse 120/min. There were oedema, exudation, and crusting of eyelids and perioral region; there were vesicular and erosive lesions of the mucous membranes of the mouth. Large denuded erythematous and exquisitely painful areas were present on her face, chest, back and legs. Flaccid bullae and purpuric macular lesions were scattered on the extremities. The Nikolsky sign was positive and the epidermis could be easily removed in large sheets (Fig. 1, 2).

Laboratory data

The Hb was 9·5 g., haematocrit 28%, W.B.C. 8800/mm³, with a normal differential count. Blood urea nitrogen, glucose, platelet determination, rapid plasma reagin card test, serum electrolytes, chest x-ray, electroencephalogram and examination of the cerebral spinal fluid were all normal. The serum albumin was mildly depressed and the γ-globulin was 0·49 g% (normal: 0·62–1·20). Cultures from blister fluid, denuded skin, the nasopharynx, anterior nares, throat, vagina, conjuntiva, blood and spinal fluid revealed no bacterial growth. The bowel flora was normal.

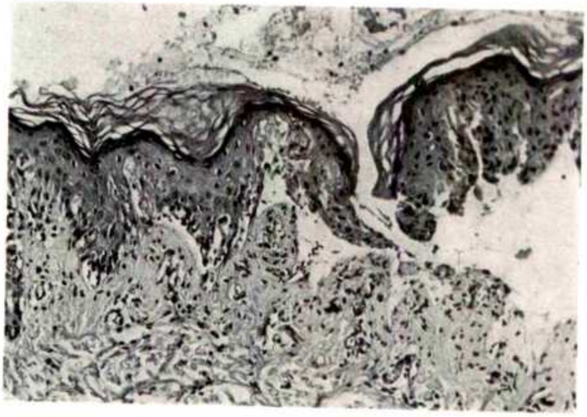

FIG. 3.—Biopsy from Case 1 reveals an epidermal–dermal separation and a large subepidermal bulla. The dermis is oedematous and contains a mild perivascular lymphocytic infiltrate (H. and E., × 130).

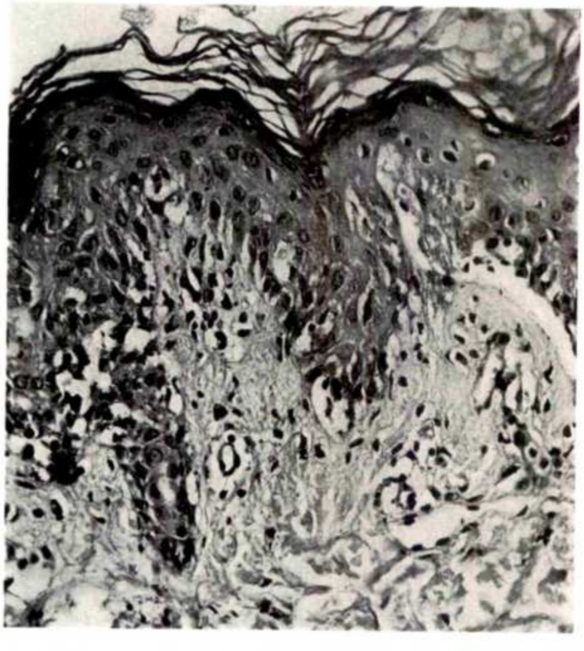

FIG. 4.—Biopsy section from Case 2 shows vacuolation of cells and spongiosis in the lower part of the epidermis, with degeneration of the basal cell layer and cleavage formation (H. and E. × 260).

A skin biopsy showed a bullous process with minimal inflammation. Areas of vacuolization and spongiosis, with degeneration of the basal cell layer of the epidermis, were also present. Small vesicular areas were seen in the lower part of the epidermis. At 1 edge of the specimen a subepidermal bulla was present. The dermal papillae

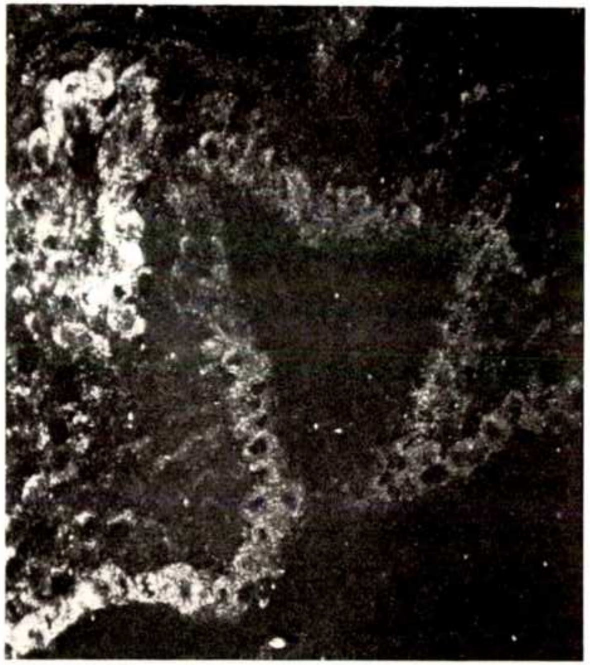

FIG. 5.—Direct immunofluorescent staining for IgG (Case 1), demonstrating intercellular immunofluorescence, confined mainly to the basal cell layer of the epidermis.

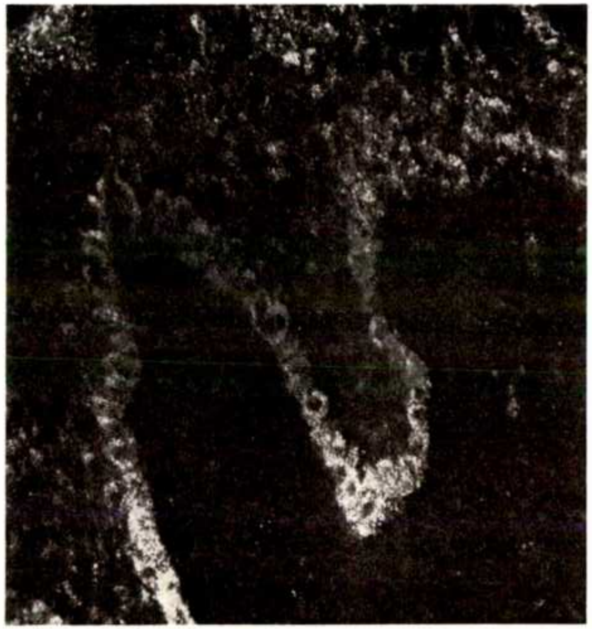

FIG. 6.—Direct immunofluorescent staining for (C^{1}3) complement (Case 1), demonstrating intercellular immunofluorescence in the basal cell layer of the epidermis.

formed the floor of this bulla, and were well preserved. The dermis was markedly oedematous. Many dilated blood vessels in the upper portions of the dermis were surrounded by a mild lymphocytic infiltrate (Fig. 3, 4). The diagnosis was bullous toxic dermatosis, consistent with a drug eruption (Dr Margaret Gray Wood).

Immunofluorescent studies

Material and methods.—Incisional biopsies were obtained from the clinically involved skin of both cases, quick frozen in liquid nitrogen, embedded in OCT media, sectioned at 4 μm in a cryostat at 20°C, and placed on glass slides. These were washed in phosphate buffer at pH 7·2 for 5 min, fixed in acetone for 10 min and air dried.

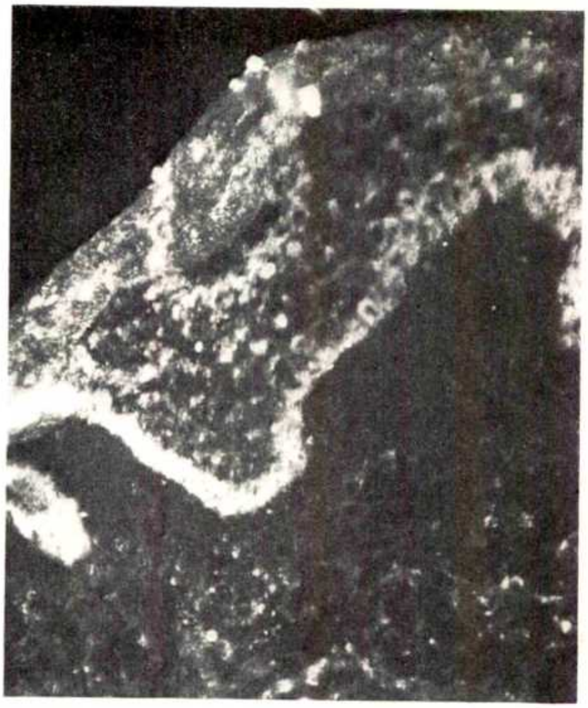

FIG. 7.—Direct immunofluorescent staining for IgM (Case 1) demonstrating intercellular fluorescence in the basal cell layer of the epidermis.

For the direct immunofluorescent studies specimens were overlaid with goat fluorescein-conjugated antisera to human IgG, IgA, IgM and globulins β_1–C/β_1A in Case 1 and to IgG in Case 2. These antisera were obtained commercially.* (They were diluted 1 : 4 with phosphate-buffered saline containing 2% Tween-80 ®.) After incubation for 30 min at 37°C, the specimens were given 3 10-min washes with phosphate-buffered saline and a final rinse with distilled water. The slides were then cover-slipped, using FA mounting media, and examined with a Leitz Ortholux microscope with a BG 12 Schott exciter filter and a K530 Leitz barrier filter.

Sections for indirect studies consisted of horizontally cut fresh human epidermis sectioned at 4 μm. These were overlaid with the patient's sera diluted 1 : 10 with phosphate buffered saline, incubated for 30 min at 37°C and washed 3 times for 10 min in phosphate-buffered saline. The remainder of the procedure was the same as described for the direct method.

Normal controls for the direct studies consisted of using fresh human skin processed as described for the direct studies. Controls for the indirect studies consisted of using normal human serum and human positive sera of patients with pemphigus foliaceous, bullous pemphigoid and systemic lupus erythematosus on horizontally cut 4 μ sections of fresh normal human epidermis.

Results.—Distinct intercellular fluorescence was seen, confined mainly to the basal cell layer of the involved epidermis with the anti IgG, IgA, IgM and the β_1–C/β_1A conjugates in Case 1 (Fig. 5, 6, 7). A similar pattern was seen in Case 2, using the anti IgG conjugate only.

Direct control studies were negative.

Indirect immunofluorescent studies were negative in both cases, using the same antisera. All indirect immunofluorescent controls were functioning properly.

Hospital course

As the patient continued to deteriorate, intravenously administered hydrocortisone was started in a daily dosage of 20 mg/kg. Intravenous fluids were given and 0·5% silver nitrate compresses with polyethylene sheeting were applied and changed every 12 hr. Striking improvement occurred within 2 days. The febrile, toxic and bullous responses were rapidly suppressed within 96 hr. One week later the hydrocortisone was changed to prednisone in a daily oral dose of 20 mg. The skin lesions had completely healed 2½ weeks after initiation of corticosteroid therapy. Prednisone was then stopped. The patient had remained in good health after surveillance for 5 months.

Case 2.—A Caucasoid male aged 21 was admitted to Philadelphia General Hospital for evaluation and treatment of a progressive bullous eruption of 1 week's duration. The patient had been maintained on chlorpromazine for psychotic episodes, and on 2 occasions therapy had had to be interrupted because of a maculopapular exanthem, which promptly cleared on stopping the medication. During the week before admission he developed a cough, coryza, low grade fever and spreading bullous lesions which involved most of his skin.

The patient appeared acutely ill, with a temperature of 39·5 °C, respiration rate 30/min and a pulse of 120/min. Large areas of his chest, back, neck and extremities were denuded of skin, erythematous, and extremely tender on palpation. Many flaccid bullae and erythematous macular lesions were present on the face, arms and legs. Vesicular and crusted erosions involved the mucous membranes.

Laboratory data

On admission the Hb was 15 g% and the white blood count 3000/mm^3, with 72% neutrophils and 28% lymphocytes. Skin, urine and blood cultures were sterile.

Skin biopsies confirmed the diagnosis of toxic epidermal necrolysis, viz. " severe degeneration of epidermal cells and subepidermal bullae with minimal inflammatory response in the dermis ". Indirect immunofluorescence was negative for circulating antibodies. Direct immunofluorescence studies on 3 occasions revealed intercellular fluorescence (IgG) confined to the basal cell layer.

Hospital course

Intravenously administered methylprednisone in a daily dose of 400 mg was instituted and maintained for 10 days. Dressings saturated with 0·5% silver nitrate were applied continually every 4 hr to the denuded areas. The dressings were changed every 12 hr. Complete resolution of the skin lesions occurred, and the patient has remained well after 2 years of follow-up.

DISCUSSION

Circulating and skin bound antibodies have been demonstrated by immunofluorescent methods in pemphigus, bullous pemphigoid (Bean, 1971; Jablonska,

1970) and lupus erythematosus (Burnham, 1970). Indirect immunofluorescent studies have revealed circulating epithelial intercellular antibodies in 2 patients with morbilliform eruptions to penicillin (Fellner, 1970) as well as in 2 patients with severe TEN (Beutner, 1970). In these cases direct studies were not recorded. The positive direct immunofluorescent studies in our 2 cases indicate intercellular fixation of immunoglobulins and complement in the basal cell layer. This discovery is analogous to the investigations of Shelley *et al.*, who recently demonstrated direct intercellular immunofluorescence confined to the basal cell layer and the 2–3 adjacents—suprabasilar cell layers of the involved epidermis in a patient with a fixed bullous eruption due to phenolphthalein (Shelley *et al.*, 1972). It is postulated that these 2 patients became sensitized to an epidermis altered by the attachment of a drug. Penicillin and chlorpromazine, or a metabolic degradation product of these drugs, may have a strong affinity for the basal cells. These drugs would then bind to protein in the intercellular space or on the surface of the cells. The resultant induction and binding of specific antibodies and fixation of complement with ensuing immunologically mediated damage would then explain the location of the microscopic and the immunofluorescent findings in this report. Certainly further investigations, using direct, in addition to indirect, immunofluorescent techniques, should be utilized in cases of bullous drug eruptions and drug-induced toxic epidermal necrolysis to study further the suspected immunological sequence of events.

Dr Herbert Goldschmidt and Dr Harold B. Heiss provided technical assistance. Mr Edward Glifort prepared the photographs.

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ROSACEA-LIKE DERMATITIS: CONTRAINDICATION OR INTOLERANCE REACTION TO STRONG STEROIDS

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Summary.—Data for 9 selected patients were used to show that rosacea-like dermatitis (RLD) is to be regarded as an intolerance reaction of seborrhoeic skin to topically-applied strong corticosteroids, such as betamethasone valerate and fluocinolone acetonide. A comparison of the geographical ranges of RLD and the use of strong corticosteroids shows that, in all countries considered, strong corticosteroids had been introduced long before RLD was observed. RLD can be cured by use of a powder suspension that is free from corticosteroids or fatty substances.

PERIORAL dermatitis or rosacea-like dermatitis (RLD) is a dermatosis known to most dermatologists (Götz and Reichenberger, 1968; Hjorth *et al.*, 1968; Kaminsky *et al.*, 1966; Kaufmann, 1965; Mihan and Ayres, 1964; Steigleder and Strempel, 1968; Steigleder, 1969). There is unanimity of opinion on the clinical course of RLD, its increased incidence, and its frequent resistance to therapy. Most authors consider its aetiology to be unknown.

This paper concerns the question of whether RLD is a contraindication for the use of strong steroids or is, in fact, caused by them. For our analysis we used both clinical findings and the experimental application of strong steroids to healthy skin. We also considered the geographical extension of the incidence of RLD in relation to the first therapeutic use of such steroids in various countries. The study was initiated after we had observed a RLD that was, at first, localized atypically after the local application of betamethasone valerate (Weber, 1970).

CASE REPORTS

Case 1 (August 1969).—A woman aged 59, of seborrhoeic type. There were multiple senile keratoses on farmer's skin on the backs of both hands, and multiple seborrhoeic keratoses on the forehead and face, with degenerative senile atrophy. There was no sign of RLD. She was treated by high-speed abrasion of the senile keratoses of the hands. After epithelialization of the surface of the abraded skin, treatment with betamethasone valerate lotion was commenced. The patient returned 8 weeks later.

She had, in the meantime, used the betamethasone valerate lotion not only on the backs of her hands but also on her face—" for cosmetic reasons ". The typical features of RLD (Fig. 1) could be seen on the backs of the hands and also on the face. The treatment with betamethasone valerate was discontinued and she was given a fat-free powder suspension. The RLD was completely cured within 8 weeks, and so far has not relapsed.

Case 2 (November 1970).—A woman aged 27 of seborrhoeic type. Betamethasone valerate ointment was originally recommended for the treatment of mild psoriasis on the backs of the hands, the axillae and the left leg. There was no sign of RLD. When

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the patient came back 18 days later, the typical erythemato-papular appearance of RLD could be seen on the hands, while psoriatic lesions had largely resolved (Fig. 2). The RLD cleared when she was treated with a fat-free powder suspension.

Case 3 (September 1970).—An obese woman aged 54, of seborrhoeic type, came to the clinic because of a typical inguinal erythrasma that had been treated for several months alternately with fluocinolone acetonide and betamethasone valerate in ointment and cream bases. Particularly at the edges, i.e. the inguinal and thigh regions, erythemato-papular efflorescences were found, some discrete and others confluent. Clinically, they were like RLD (Fig. 3). The erythrasma healed when treated with antimycotics, but the RLD persisted. After local treatment with a fat-free powder suspension, slow improvement took place and the condition healed within 7 weeks.

Case 4 (September 1970).—A woman aged 39, of seborrhoeic type, with a pre-auricular scar, about 2cm long, on the right side. The area surrounding it was thickened and indurated. Within a year, a nodule had developed at the site; it was diagnosed as a basal cell epithelioma and treated with x-rays. An erosive reaction resulted, and the patient treated this with betamethasone valerate ointment for about 6 weeks. Towards the end of this treatment, " red spots, little lumps and little blisters " appeared in the region of the irradiated area. They gradually spread over both cheeks, and parts of the forehead and chin. Diagnosis: relapse of an irradiated pre-auricular epithelioma, with typical RLD. The epithelioma was excised. The RLD improved considerably within 3 weeks when treated with a fat-free powder suspension. When the patient returned for examination in February, the RLD had healed completely.

Case 5.—A woman aged 74, of seborrhoeic type, was treated daily with 1 mg of betamethasone valerate for a haemolytic anaemia, from the beginning of July until early September 1970. In the middle of August 1970 she noticed the appearance of " little red lumps, spots and pimples ", first on her back, then on the shoulder girdle, across her chest and, finally, over both cheeks. Local application of cosmetic preparations was ineffective. At the first dermatological examination on September 10th, 1970, at the instigation of the physician who was treating her, the typical efflorescences of RLD were found in great numbers on the trunk and, with less intensity, on her face. The necessary continuation of the systemic treatment with betamethasone valerate up to January 1971 led to a further extension and intensification of the RLD on her face (Fig. 4).

Case 6.—A woman aged 34, of seborrhoeic type, had been treated systemically for several months with corticosteroids for a chronic urticaria, which had appeared after hysterectomy. After an initial improvement, relapses took place on the arms. Because she did not tolerate systemic corticosteroids well, her treatment was changed; every evening for about 2 years she applied a 0·2% fluocinolone acetonide ointment.

At our first examination in September 1970, a tender atrophy of the skin was visible on both arms, to about 10 cm below the elbows, as can often be seen after a long-term application of corticosteroids. On the chin a discrete, but typical, RLD was found that had been present " for many months ". For a time she had been treating her chin also with fluocinolone acetonide ointment, but had given this up some time before when it proved unsuccessful, and had simply covered the area with cosmetics. When she was asked in detail about the possibility of her chin coming into contact with fluocinolone acetonide since then, she admitted that she had applied the 0·2% fluocinolone acetonide ointment to her arms immediately before going to bed and that she habitually slept with her chin in the crook of her left arm.

Case 7.—A woman aged 83, of seborrhoeic type, on maintenance treatment for pemphigus with ocular involvement from June 10th to November 13th, 1970. The disease slowly cleared with systemic prednisolone and topical triamcinolone cream. However, during the disease, symblepharon with entropion developed, making epilation of the lashes necessary. The eyelids were treated locally for several weeks with betamethasone valerate cream; a typical RLD developed, beginning on the eyelids and spreading over both cheeks as far as the nasolabial fold and the upper lip. The RLD cleared up, although slowly, after discontinuation of the steroid ointment and initiation of treatment with fat-free powder suspension.

Case 8.—A woman aged 60, of seborrhoeic type, with a gravitational leg ulcer and pernicious anaemia. In addition to the routine treatment of the leg ulcer, the patient's tolerance was tested by the application of betamethasone valerate cream every day to the healthy skin of the left cheek for more than 4 weeks (Fig. 5). Fourteen days later, an additional daily treatment of the left eye with a dexamethasone ointment became necessary because of a previously known glaucoma. In the fourth week of application of betamethasone valerate, RLD of increasing intensity appeared. It was localized principally above the left cheekbone and spread over the left cheek, the left eyelid and the bridge of the nose, and later over the whole of the left side of the face (Fig. 6). After use of betamethasone valerate cream and dexamethasone ointment had been discontinued, the RLD healed within 8 weeks when treated with a fat-free powder suspension. There were several relapses, each of short duration.

Case 9. (November 1970).—A boy aged 11 months. Betamethasone valerate cream was used locally to treat an haemangioma in the occipital area. When he was brought back for examination in March 1971 the angioma was clearly beginning to disappear. The skin of both cheeks, however, which had been free of pathological changes in November 1970, now displayed a typical RLD. The mother had used betamethasone valerate cream to soothe the skin of his cheeks.

DISCUSSION

The 9 cases summarized above are only a small proportion of the 113 patients with RLD that we have observed. Their number is steadily increasing. Selection of the patients for this report was guided by the need to follow exactly the course of the disease and to define all the circumstances that could be held responsible for causing it. In all the patients specifically questioned on this subject last year, the RLD had been treated topically or systemically with corticosteroids. In most cases, various corticosteroids had been applied sequentially; these were mostly of fluorinated type, usually betamethasone valerate or fluocinolone acetonide, and, less often, clocortolone capronate, dexamethasone, or triamcinolone acetonide in various formulations. A comparison of the compositions of the various bases of the creams and ointments showed no component common to all the vehicles. It is, therefore, less probable that the cause of RLD is intolerance to the vehicle.

The relationship between the development of RLD and the use of the corticosteroids was obvious. Patients were surprised when the doctor was able to tell them they had been treated with corticosteroids. However, it was often difficult to determine the date of onset of the RLD and of the first application of a corticosteroid, partly because of the patient's inability to describe the first skin changes and partly because these changes often occurred several years before probably, therefore, forgotten. Very often the first corticosteroid treatment was introduced not for RLD but for " roughness ", skin that was too " dry ", or for " eczema ", and sometimes for the treatment of telangiectases or for " pimples ". For some patients these ointments and creams were borrowed from another member of the family who was being treated by a doctor. Only 3 patients admitted that they had treated themselves in this way for the present RLD that had " appeared spontaneously ". Like Steigleder and Nasemann we observed an overwhelming preponderance of seborrhoeic types, even allowing for the difficulty of diagnosing this state in children. The condition occurs more in middle-aged women than in men, and more in men than in children. There is no justification for the assumption that oral contraceptives are of any aetiological significance (Ehlers, 1969; Götz and Reichenberger, 1968).

Use of corticosteroids did not lead to a cure in any of the cases we observed, but it did produce a temporary improvement, which was nevertheless followed by a progression of RLD. In all the cases we treated the application of a fat-free powder suspension, with simultaneous imposition of a " no-washing " rule, led, after obvious initial deterioration in the first 2 weeks, to a cure. The additional oral administration of tetracyclines speeded up the healing process in those cases in which pustular lesions were also present. Tetracycline therapy did not produce a cure if the topical corticosteroids treatment was continued, nor did tetracycline treatment alone.*

These observations show that these cases of RLD had all been treated unsuccessfully with corticosteroids and can be healed by the application of a fat-free powder suspension. The development of RLD appears to require a seborrhoeic type of skin (a " greasy skin ") as an essential factor (Steigleder and Strempel, 1968; Weber, 1971). In the German literature the seborrhoeic type has been defined and described by Unna in 1884. It concerns a high secretion of sebum measurable in the areas of the skin rich in sebaceous glands. The replacement time of skin sebum is shorter in seborrhoeic types than in the dry-skinned (Kleine-Natrop, 1957; Weber, 1971).

Cases 1–8 cited above, which were selected from the wide range of affected individuals available to us, are all characterized by a seborrhoeic type of skin. These cases are particularly notable in that RLD had not appeared in any of them before the application of strong corticosteroids. The time factor in Case 1 is of interest. Here we observed that local treatment with betamethasone valerate, in conjunction with the high-speed abrasion of senile keratoses, caused RLD on the backs of the hands and on the face. The same thing happened in Case 2, although not so dramatically; here it concerned a psoriasis vulgaris, in which the first local application of betamethasone valerate induced RLD. In Case 3, an inguinal erythrasma, only betamethasone valerate and fluocinolone acetonide were administered for a long time. RLD-like phenomena appeared, especially at the edge of the erythrasma. They persisted after the erythrasma had healed, despite antimycotic treatment, and did not heal until a fat-free powder suspension had been applied. They cannot, therefore, be regarded as directly related to the erythrasma. In Case 4 also, contact with betamethasone valerate was undoubtedly made before the RLD appeared. The phenomena here developed from an erosive reaction to x-ray, which was first treated with betamethasone valerate.

The patient treated systemically for months with betamethasone valerate because of haemolytic anaemia (Case 5) is a particularly impressive case. The steroid-acne was similar to that which we are used to seeing, for example, with prednisolone or methylprednisolone. However, the eruption on this patient's back and cheeks was different from steroid-acne (Steigleder, 1969) in that there were no comedo-like hyperkeratoses and no closure of the follicular ostia. The lesions were characterized by raised erythema with pustules and discrete small red papules, corresponding morphologically to typical RLD. Topical treatment and cosmetic preparations had not been used. What is remarkable is that continuation of the systemic treatment with betamethasone valerate produced an intensification of the RLD on the face (Fig. 4). Although the systemic use of betamethasone valerate had here led to the RLD, Case 4 possesses the classical characteristics of a " contact dermatosis ". The patient had been treated for a long time with fluocinolone acetonide for papular urticaria of both arms, and the RLD appeared on her chin, i.e. in a typical localization for RLD. The *prerequisite* was seborrhoea of the face; the *cause* was contact during the night between the chin and the crook of the arm that had previously been regularly treated with fluocinolone acetonide. The administration of the fluocinolone acetonide ointment had not produced RLD in the skin of the upper arm, which had fewer sebaceous glands and was also (because of the steroids) atrophic. Cases 7–9 all offer equally convincing evidence that RLD arises as a result of the use of strong corticosteroids. In Case 7 the application of betamethasone valerate to treat pemphigus caused typical RLD of the face. In Case 8 a 4-week administration of betamethasone valerate and a 2-week application of dexamethasone cream to *healthy* seborrhoeic facial skin produced RLD of the face. In Case 9 " care " of the healthy skin of a child with betamethasone valerate led to RLD.

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* Kirton *et al.* (in preparation) have observed a satisfactory response to oral tetracycline therapy, provided topical steroids are strictly avoided (Editor).

TABLE I.—*Relationship of Use of Fluocinolone Acetonide and Betamethasone Valerate to Occurrence of Rosacea-Like Dermatitis**

Year of initial publications on rosacea-like dermatitis	Year of initial therapeutic trials with fluocinolone acetonide (F) or betamethasone valerate (B)	Time of initial marketing of fluocinolone acetonide
USA 1964 (20) 1965 (15)	USA F: 1960 (22, 27) 1961 (30) 1962 (17, 18) B: 1964 (19)	USA April 1961
South America 1966 (14)	Mexico B: 1964 (21)	
Great Britain and Scandinavia 1968 (12, 34)	Great Britain B: 1962 (35) F: 1962 (26, 23) B+F: 1964 (5) B: 1964 (39, 24) B+F: 1965 (6)	Great Britain April 1961
	Scandinavia F: 1962 (1) B: 1963 (2) B: 1964 (3, 11)	Scandinavia Sweden: Jan. 1962 Finland: Feb. 1962 Denmark: July 1962 Norway: July 1962
West Germany 1968 (10, 32)	West Germany F: 1962 (4) 1966 (28) B+F: 1966 (29)	West Germany November 1962
East Germany None	East Germany None	East Germany Not marketed

* References shown in parentheses.

These findings prove that the administration of strong corticosteroids such as betamethasone valerate, fluocinolone acetonide, and perhaps also dexamethasone, to those areas of the skin that are rich in sebaceous glands can cause RLD.

An important prerequisite appears in our opinion to be the presence of a seborrhoeic diathesis. It must be said, however, that not every seborrhoeic patient will be afflicted with RLD after using these corticosteroids. Not everyone who is treated systemically for a long time with corticosteroids becomes afflicted with a steroid-acne that is indisputably an intolerance reaction to a corticosteroid. For this reason it seems to us that there are also other, still unknown, factors that are prerequisites for both steroid-acne and RLD. Our opinion as to the cause of RLD is further supported by the fact that corticosteroids are not able to heal RLD (Götz and Reichenberger, 1968; Sneddon, 1968; Wilkinson, personal communication; Winkler, 1970), but tend to make it worse. We have also encountered the objection that the RLD, according to the patient, appeared before the first application of these steroids. These patients were not in a position to remember the exact type of the primary skin symptoms, nor could they describe accurately the changes that developed. This shortcoming can be attributed to the patient's lack of technical knowledge and to the fact that it is difficult to remember the morphological development of a disease that often lasts a long time.

If we now ask ourselves about the reasons for the rising frequency of RLD and about its geographical range, we find that there are parallels here, too, between RLD and the use of these corticosteroids. Table I shows, as exemplified by fluocinolone acetonide and betamethasone valerate, a parallelism in the geographical range of RLD and the introduction of these corticosteroids into the dermatological therapy of these countries. It can generally be confirmed that the first publications on RLD in the U.S.A., Great Britain, Scandinavia and West Germany appeared years after the first publications on the clinical use of the named corticosteroids. It can be seen even more clearly that these corticosteroids were always forerunners of RLD, usually by a long period. Frumess and Lewis (1957) seem to have found an exception in their observation on the light-sensitive seborrhoeide that was identified by Mihan and Ayres (1964) as a RLD. As we see it, the cyclic course, the absence of pruritus, and the fact that the condition was cured when treated with chloroquine derivatives, which we did not observe when we treated RLD, all suggest that this disease was not RLD. In addition, we did not observe the presence of telangiectasia in RLD, in contrast to the light-sensitive seborrhoeide (Frumess and Lewis, 1957).

Our case observations, the relationship between the geographical extent of RLD, the range of the named corticosteroids, and the worsening effect of the corticosteroids on RLD are indisputable arguments for the view that RLD is an intolerance reaction of the seborrhoeic regions of the skin to strong corticosteroids, just as steroid-acne is an intolerance reaction to systemically administered corticosteroids. From this report it can be seen that the administration of strong corticosteroids to seborrhoeic patients, at least on the face, should be avoided.

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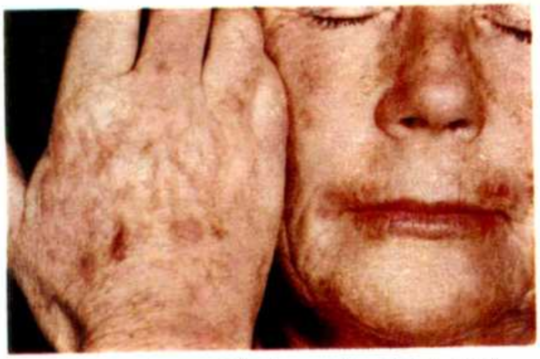

FIG. 1.—Rosacea-like dermatitis after topical application of betamethasone valerate.

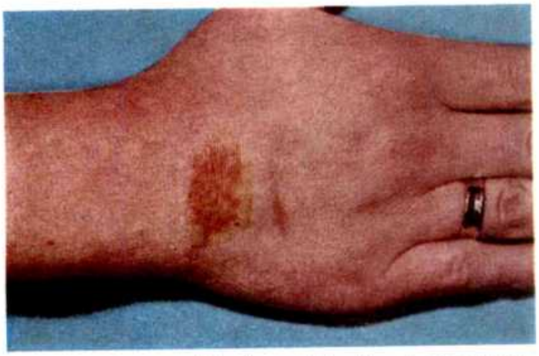

FIG. 2.—Rosacea-like dermatitis after topical treatment of psoriasis vulgaris with betamethasone valerate.

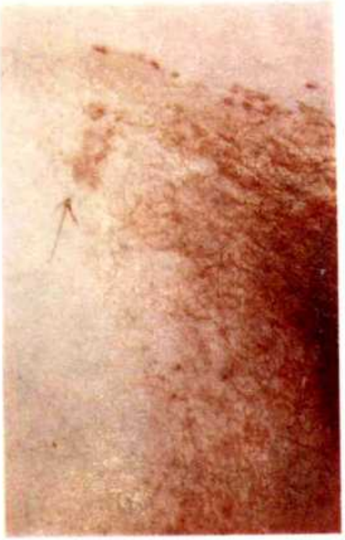

FIG. 3.—Rosacea-like dermatitis after treatment of erythrasma with betamethasone valerate.

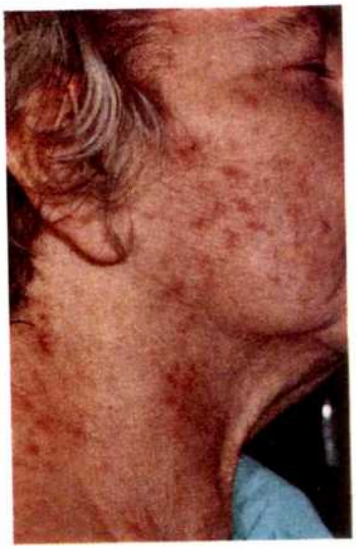

FIG. 4.—Rosacea-like dermatitis after systemic therapy with betamethasone valerate.

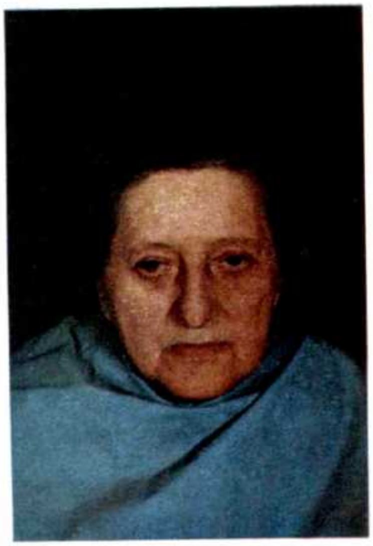

FIG. 5.—Prior to topical treatment with strong corticosteroids.

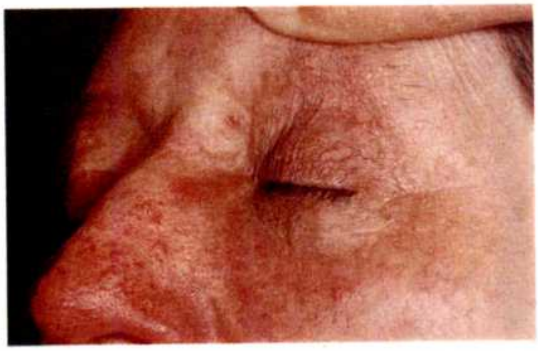

FIG. 6.—Rosacea-like dermatitis 4 weeks after topical treatment with strong corticosteroids.

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SKIN MARKINGS IN NEGRO CHILDREN FROM THE WEST INDIES

DOROTHY I. VOLLUM

Summary.—Three hundred and forty-six West Indian children aged 0 to 11 were examined for mongolian spots and Voigt's lines. The incidence of mongolian spots showed a linear drop with age; they were present in 95% of children under a year and 3% of children aged 10. Thus they are common in children over a year. Linear differences in pigmentation along the anterior border of the arm were found in 26% of the 235 children.

Most West Indian infants of negro extraction have slatey blue macular areas of pigmentation in the sacral area, known as mongolian spots. They may also have sharply demarcated lines between the darker dorsal and the paler ventral surfaces of the arm, which are sometimes named after the Viennese anatomist, Voigt.

In a recent report (Moynahan, 1970) it was stated that nearly all mongolian blue spots fade within a few months of birth and that, exceptionally, they may last for a year or longer. Experience with the West Indian negro population in London showed that it was fairly common for these spots to be present in older children; and, in order to confirm this impression, 346 children up to the age of 11 were examined and the incidence of the spots at the various ages was calculated. At the same time, the presence or absence of Voigt's lines was recorded.

Mongolian spots are found in infants of mongoloid and negroid races at birth, and occasionally in Caucasoids. They vary from about 1 to 14 or more cm in diameter. They are commonest in the sacral area and on the buttocks. They are often present on the back and shoulders, less frequently on the arms and legs, and rarely on the face. They may be single or multiple. Sometimes several spots coalesce so that large areas of the back may be blue.

Histological examination of the lesions shows elongated melanocytes lying between the collagen fibres of the dermis running parallel to the skin surface. There is no disturbance of the pattern of collagen or elastic fibres, and melanophages are not present. The blue-grey colour is due to a particular optical relationship between collagen and melanin in the dermis, which leads to subtractive colour mixing (Findlay, 1970). The dermal collagen reflects the shorter blue wavelengths of white light, while the deeper melanin absorbs the longer green to red wavelengths, and the affected area appears blue. In the embryo there has been a failure of some of the melanocytes to migrate from the neural crest through the dermo-epidermal junction. The completion of this process causes the spots to disappear.

Voigt's name was first used by Matzumoto (1913) to describe the line which can sometimes be seen marking a striking difference in pigmentation between the dark

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extensor and paler flexor surfaces of the arms. This corresponds with the anterior axial line which marks the junction of the dermatoses or areas of skin supplied by individual spinal nerves. This runs from the level of the second costal cartilage on the sternum, laterally over the pectoralis major, down the anterior border of the upper arm and then medially across the flexor surface of the forearm to the mid point of the wrist.

PATIENTS AND METHODS

Three hundred and forty-six children up to the age of 11 were examined for mongolian spots. About 75% were attending the clinic with atopic eczema, ringworm or impetigo, and the rest were fit children seen at routine school medical examinations. Only negro children whose parents were born in the West Indies were examined, and the mother's island of origin was recorded as the child's nationality. The children themselves were nearly all English by birth. Ethnically they were probably of mixed African Negroid and Caucasoid origin.

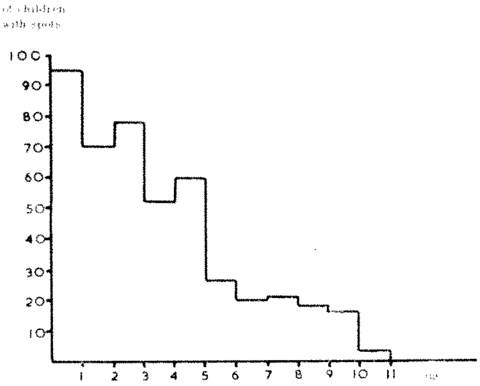

Fig. 1.—Histogram to show the decreasing incidence of mongolian spots with age in 346 West Indian children.

RESULTS

Of the children 91 % were Jamaican, 6 % came from Barbados and the rest from Dominica, St. Lucia and St. Vincent; 38 % of the children had Mongolian spots. There was no sex difference in incidence—52 % were in males and 48 % in females. It was commoner to have multiple than single spots (Fig. 2). Approximately 66 % of the children had more than 1 spot. The ratio of children with multiple spots to those with single did not alter with age ($\chi^2 = 5{\cdot}0$ with 10 degrees of freedom). Thus, multiple spots did not persist longer. The commonest sites were the sacrum and buttocks—only 3 of the 132 children did not have them there. They were less frequent on the shoulders, back and arms. A few were seen on the legs and only 1 on the face. There was a linear drop in incidence with age—95 % of the children under a year had spots, but only of 3 % children aged 10 to 11 (Fig. 1). χ^2 done on Table I to test whether the incidence is constant with age = 111·5 with 10 degrees of freedom, $P < 0{\cdot}0005$. Thus it is very unlikely that the observed decrease in incidence with age is due to chance.

Two hundred and thirty-five children were examined for Voigt's lines, and they were found in 26 % (63); 46 % were females and 54 % males. In 3 % (8) the line

TABLE I.—*Table Showing the Number of Children with Spots in the Various Age Groups*

	Age group											
	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	Totals
No spots	1	8	7	12	11	20	27	33	36	31	28	214
Spots	20	19	25	13	17	7	7	9	8	6	1	132
Total	21	27	32	25	28	27	34	42	44	37	29	346
% with spots	95	70	78	52	60	26	20	21	18	16	3	38

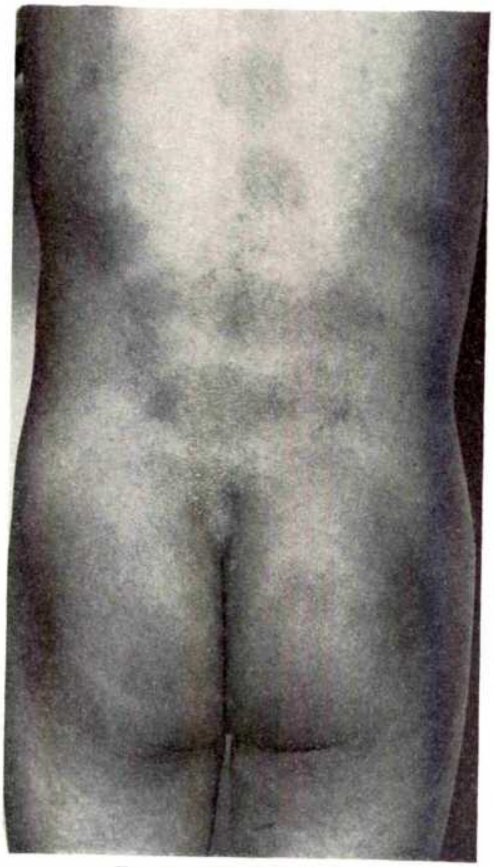

FIG. 2.—Mongolian spots.

was present on only 1 arm; they were not seen on the legs. In the West Indians the lines were most marked on the upper arm. Proximally they faded out over the head of the humerus. Distally they ran on the anterior border of the arm to the lateral side of the base of the first metacarpal, often becoming indistinct below the elbow. The incidence in the various age groups increased slightly with age, but this increase was not significant ($\chi^2 = 9{\cdot}4$, $P > 0{\cdot}5$). Colour differences were occasionally seen on the back of the upper arm following the dorsal axial line.

DISCUSSION

Mongolian spots in the West Indians are common in children over a year. Their disappearance follows a linear drop with age so that they are still found in 16 % of children aged 9. A survey of 600 Japanese children (Kato, 1905) showed them still present in 6 % of children aged 9. In Sardinia, where 2588 children were examined (Filia, 1916), only 63 had mongolian spots and none were found in children over 5. It is important to be aware that mongolian spots do occur in older children, as they may occasionally be confused with bruises. However, their persistence several weeks after the supposed trauma, their distribution and slatey-blue colour should make the diagnosis obvious. The differential diagnosis from blue naevi and the naevus of Ota and Ito is important only prognostically. Blue naevi are usually palpable and they persist throughout life. The naevus of Ota, in the distribution of the trigeminal nerve on the face, or that of Ito on the shoulder, can usually be diagnosed by the site.

Voigt's lines in the West Indians follow a slightly different pattern from those in the Japanese. In the latter, they follow exactly the anterior axial line of the arm, and the pigmentary differences are also present along the dorsal axial line and the axial lines of the legs, suggesting a relationship between pigmentation and innervation. In the West Indians they did not follow the axial line, passing laterally to it on the forearm. In most cases the lines were probably less obvious than those in the Japanese. The incidence was also higher. Ito (1965) found them in only 4·3 % of 3000 Japanese, whereas they were present in 26 % of 235 West Indians. In the Japanese they were 10 times commoner in females, whereas in the West Indians the sex incidence was relatively constant.

I am indebted to Dr R. P. Knill-Jones for the statistical calculations, to Dr A. L. Thrower for allowing me to examine children at routine school medical examinations, and to Dr D. I. Williams and Dr R. H. Marten for allowing me to examine their patients.

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THE INVESTIGATION OF PHOTOCONTACT DERMATITIS IN A DYE MANUFACTURING PROCESS

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Summary.—An investigation of photocontact dermatitis occurring in a dye manufacturing process is described. Sixteen employees were studied by photopatch testing to the complete anthraquinone-based dye: Disperse Blue 35 and to chromatographically separated bands. By irradiation of the skin with visible light between 400 and 700 nm evidence was obtained to show that the complete dye produced photo-contact dermatitis in individuals, whether they had previously been exposed to it or not. Using the same technique, only 4 of the 10 constituent bands produced a similar photocontact reaction.

In part of a chemical factory manufacturing an anthraquinone-based dye "Disperse Blue 35", employees had suffered during the summer months from erythema and discomfort of the exposed skin of the face, neck and hands. It was therefore decided to try to determine the cause of this photocontact reaction and thus to establish which aspect of the industrial process might be responsible. Initial photopatch testing carried out at the factory, using an unfiltered 500 W xenon arc light source, failed to produce confirmatory positive photopatch tests. This failure was probably due to the erythema produced by the short sunburn waveband preventing a satisfactory assessment of the long ultra violet (*u.v.*) and the visible parts of the spectrum. The opportunity was therefore taken to investigate dye process workers, using once again a xenon light source, but this time coupled to a diffraction grating monochromator to facilitate selection of appropriate narrow wavebands in the visible wavelengths.

Clinical Features

During the last 10 years one of us (J.S.G.) had been consulted by approximately 130 employees with abnormal skin reactions on the face, neck, hands and forearms, consisting essentially of erythema and swelling of the skin, sometimes lasting 2 or 3 d, but not infrequently for 24 h only. Most of the workers affected were on a 3-shift system, i.e. 6 a.m.–2 p.m. (day shift), 2 p.m.–10 p.m. ("back" shift), and 10 p.m.–6 a.m. (night shift). The majority of cases occurred in workers on the day shift, who developed skin discomfort soon after leaving the factory on bright sunny days, and even whilst on their way home. Although evening sun did not usually cause symptoms, occasionally in the early evening exceptionally bright sunlight would do so. Those day shift workers who had burning and erythema in the afternoon were usually compelled to stay indoors or at least out of direct

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sunlight. The condition affected both new and long-term employees. Most affected personnel noticed that the sun made their skin react, even when it was transmitted through window glass, such as when they were driving their cars. Although many employees were affected at some time or other, a significant occupational problem had not arisen, since the response of the skin was relatively short-lived, and loss of working time was unusual.

METHOD

A total of 16 male employees, ranging in age from 22 to 57 years, was studied. Initially, the complete dyestuff (Disperse Blue 35) was used for testing. Disperse Blue 35 is made from 1 : 8 dihydroxy anthraquinone by a process of nitration, reduction and methylation, no single stage of which is completed, the final product therefore containing a mixture of the various partially-reacted intermediates. Since a dyestuff such as this is not a pure chemical, and since a standard method of analysis in the industry is that of thin layer chromatography, the photopatch test study was enlarged to include samples of chromatographically-separated bands of the dyestuff to identify which one might be responsible for the photocontact reaction.

TABLE I.—*Maximal Absorption Peak for Each of the Constituent Bands of Disperse Blue* 35

Chromatograph fraction	Absorption peak (nm)
Band 1	428
2	670
3	625
4	650
5	560
6/7	580
6/7	420
8	620
9	575
10	546

The technique used involved preparing a thin layer of suitable adsorbent (silica gel) on a 4 mm thick glass plate and drying. The material to be analysed was then applied to the edge of the adsorbent layer and the plate placed in a suitable glass tank containing approximately 0·25 in layer of a suitable solvent.

The chromatogram develops rapidly by capillary rise of solvent up the layer; the individual components of the sample migrate at different speeds and are thus separated. In the case of Disperse Blue 35, 10 different bands (numbered 1 to 10) were identified. Bands 6 and 7 did not separate sufficiently to allow their removal individually. The concentrations of these bands and of band 5 were small, and it was not possible to recover from a series of chromatograms a sufficient supply for use in patch and photopatch testing. The maximum absorption peak for each band was estimated spectrophotometrically, using a Unicam SP800 spectrophotometer (Table I), and the scans of each band are shown in Fig. 1. Also included was the immediate precursor (I.P.), which consisted of a mixture of bands (numbers 1, 5, 7, 8, 9 and 10), and to which the employees were exposed at 1 stage in the manufacturing process. A maximally-concentrated solution of the dye in acetone was applied to the skin of the back as a patch test consisting of lint covered by " Blenderm " tape, black polythene and " Micropore " tape. A series of such patch tests was read at 48 h to exclude delayed type allergic sensitivity. A second series of similar patches was used as photopatch tests, this series being irradiated 24 h after application. The source of irradiation was a 1·6 kW xenon

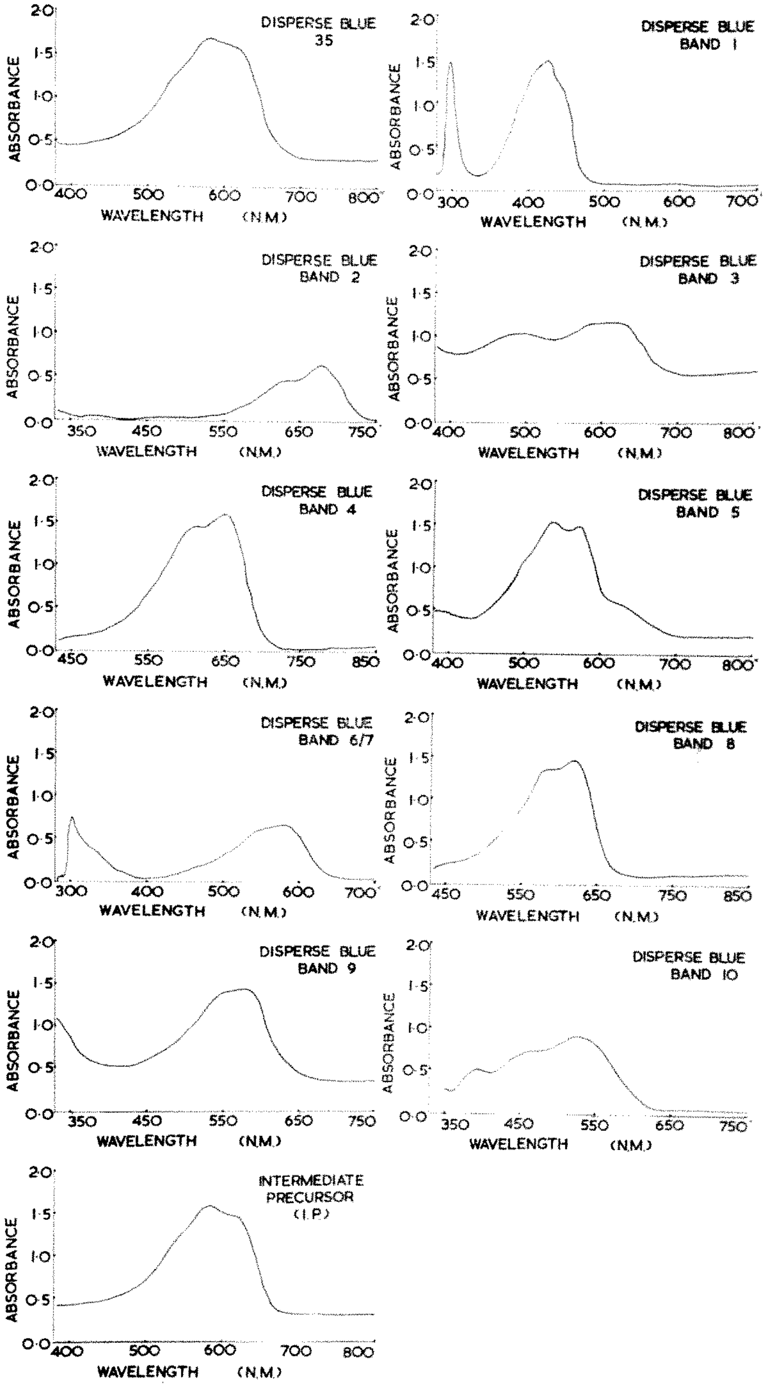

FIG. 1.—Absorption spectra of the complete dye (Disperse Blue 35) and of its 10 constituent bands and its immediate precursor state (I.P.) (acetone was used as the solvent, the concentrations being those most suitable for absorbance recording).

arc, the emitted light being passed through a diffraction grating monochromator (Bausch and Lomb 33–86–48), which allowed a selection of precise spectral bands with a half band width of 30 nm. The wavebands were chosen on the basis of the absorption of the dye components (Table I). Light scatter and second order spectra were minimized as far as possible by the use of Schott filters, i.e. WG1 at 420, 428 nm; OG4 at 546, 560, 575, 580, 620, 650 and 670 nm. The energy output at each band pass was measured in mJ/cm^{-2}, using a Hilger and Watt thermopile ($FT17SiO_2$) connected to a Solartron digital voltmeter. The presence or absence of an erythematous response was noted at 7–8 h, 24 h and 48 h. The erythemal doses were established and the following grading used:

0 = no erythema
0·5 = doubtful erythema
1 = faint but definite erythema
2 = marked erythema
3 = marked erythema with minimal oedema
4 = marked erythema with marked oedema.

TABLE II.—*Results Obtained on Photopatch Testing the Original Subject (J.H.) to the Complete Dye at Wavelengths between* 400 *and* 700 *nm*

Name	Date of test	Wavelength peak (nm)	Type of phototest	Dose (J/cm^2)	Result* 7 h	Result* 24 h
J.H. (Case 1, Table IV)	December 1968	600	Untreated skin	25	0	0
				50	0	0
		600	Complete dye	25	1	1
				50	3	3
J.H.	March 1969	420	Complete dye	78	0	0
		546	Complete dye	55	0	0
		560	Complete dye	55	0	0
		575	Complete dye	51	0	0
		620	Complete dye	22	2	1
		650	Complete dye	21	3	3
		670	Complete dye	19	Petechiae	

* Numbers refer to grade of erythema.

In addition to routine photopatch testing, phototesting was performed throughout the spectral range 400 to 700 nm at the specific wavelengths already listed to determine whether photosensitivity existed other than as photocontact dermatitis.

RESULTS

The first employee to be investigated (Case 1, J.H., Table II) in December 1968, produced a positive photopatch test with the maximally-concentrated complete dye (Disperse Blue 35) following irradiation at 600 nm, which band pass had been found from spectrophotometric studies to straddle approximately the peak of the absorption spectrum of the dye (Fig. 1). Three months later, in March 1969, additional photopatch tests were carried out, again using the complete dye, but this time irradiating also with wavebands throughout the visible spectrum between 420 and 670 nm to determine whether other parts of the absorption spectrum of the dye were responsible, in addition to the already incriminated 600 nm band pass. Positive photopatch test responses were obtained only at 620 nm and 650 nm, the remainder being negative, i.e. at 420 nm, 546 nm, 560 nm and 575 nm. The petechial reaction at 670 nm (Table II) was of uncertain significance, but probably represented a positive response in that subsequent photopatch testing at 670 nm, with a highly methylated specimen of the complete dye, was positive. Routine photo-testing on untreated skin at the same wavebands showed no abnormality, thus confirming the significance of the positive photopatch tests at 620 and 650 nm, and probably also at 670 nm.

The results from photopatch testing in the same individual (Case 1, J.H., Table II), using the chromatographically-separated bands, is shown in Table III, positive responses being obtained only with band 4, 8, 9 and 10 with the immediate precursor (mixture of bands 1, 5, 7, 8, 9 and 10) at 560, 575, and 620 nm wavelength peaks; a grade 3 positive response was noted with band 2 at 7 h when irradiated at a wavelength peak of 670 nm, but this had cleared by 24 h. These results indicated that most probably the responsible wavelength lay within the visible light in the region of 500 to 670 nm, and that only some of the chromatographically-separated bands of the dye were responsible for the photocontact dermatitis. In an attempt to confirm these results, the investigation was enlarged to include the study of an additional 15 employees. At the same time the opportunity was taken to simplify the technique of investigation, since it otherwise entailed personnel being off work for 2 or 3 days for admission to hospital 60 miles from the factory.

TABLE III.—*Results Obtained on Photopatch Testing the Original Subject (J.H.) to the Constituent Bands (1 to 10) of the Complete Dye*

Name	Date	Wavelength peak (nm)	Photopatch test material	Dose (J/cm²)	Result*		Control phototest result
					7 h	24 h	
J.H. (Case 1, Table IV)	March 1969	546	Band 10	61	0	0	
		575	Band 9	57	0	0	
		620	Band 8	50	0	0	
	March 1971	546	Band 10	56	2	2	
		575	Band 9	56	2	2	
		620	Band 8	56	2	2	
	June 1969	670	Band 2	27	3	0	0
		625	Band 3	30	0	0	0
		650	Band 4	29	2	1	0
		580	Band 6/7	37	0	0	0
		420	Band 6/7	56	0	0	0
		428	I.P.	55	0 (Pet)		0 (Pet)
		546	I.P.	38	0 (Pet)		0
		560	I.P.	38	2	2	0
		575	I.P.	36	1	1	0
		620	I.P.	36	2	2	0

Pet = Petechial response.
* Numbers refer to grade of erythema.

In Table IV can be seen the results of the investigation of the additional 15 employees (Case 2–16), and also the original subject (Case 1—J.H.). In the main these results confirmed the information already obtained from the original study, i.e., in addition to the complete dye, band 4 and the immediate precursor were responsible for the photocontact reaction.

We were particularly struck by the occasional unexpected positive response, such as band 2 with Cases 5 and 1, and band 6/7 with Cases 2, 3 and 4. The combination, however, of otherwise 5 negative responses with 3 doubtful positive responses would favour the assumption that band 6/7 was not responsible for the

TABLE IV.—*Results Obtained on Photopatch Testing the* 16 *Employees to the Complete Dye and its Constituent Bands* (1 *to* 10)

Photo patch test material	Wavelength peak nm	1 J.H.	2 G.M.	3 W.P.	4 R.C.	5 W.N.	6 A.McI.	7 M.M.	8 J.G.	9 W.A.	10 E.S.	11 G.McB.	12 J.G.	13 F.N.	14 R.R.	15 S.T.	16 W.H.
Band 2	670	Pos?	Neg	Neg	Neg	Pos	Neg	Neg	Neg	—	—	—	—	—	—	—	—
Band 3	625	Neg	Neg	Neg	Neg	Neg	Neg	—	—	—	—	—	—	—	—	—	—
Band 4	650	Pos	Pos	Neg	Neg	Pos	Neg	Pos	Pos	Pos	Pos	—	—	—	—	—	—
Band 6/7	580	Neg	Pos?	Pos?	Pos?	Neg	Neg	Neg	Neg	—	—	—	—	—	—	—	—
Band 6/7	420	Neg	Neg	Pos?	Neg	Neg	Neg	—	—	—	—	—	—	—	—	—	—
I.P.	428	Pet	Neg	Neg	Neg	Neg	Neg	—	—	—	—	—	—	—	—	—	—
I.P.	546	Pet	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	—	—	—	—	—	—
I.P.	560	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	—	—	—	—	—	—
I.P.	575	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos?	Neg	Neg	—	—	—	—	—	—
I.P.	620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	—	—	—	—	—	—
Complete dye	650	Pos	—	—	—	—	Pos	Pos	Pos	Pos	Pos	—	—	—	—	—	—
Band 8	620	Pos	—	—	—	—	—	—	—	—	—	Pos	Pos	—	—	—	—
Band 9	575	Pos	—	—	—	—	—	—	—	—	—	Neg	Pos	—	—	—	—
Band 10	546	Pos	—	—	—	—	—	—	—	—	—	Pos	Pos	—	—	—	—
I.P.	6 kW xenon light source + filter	—	—	—	—	—	—	—	—	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos
Complete dye	6 kW xenon light source + filter	—	—	—	—	—	—	—	—	Pos	Pos	Pos	Neg	Pos	Neg	Pos	Pos

Pet = Petechiae.

photocontact dermatitis; and similarly with band 2, in which there were 6 negative responses, 1 doubtful positive response (Case 1), and only 1 positive reaction (Case 5).

What was more puzzling, however, was the complete lack of response in March 1969 of the original employee (Case 1—J.H.) to bands 8, 9 and 10, which are present in the immediate precursor which had already been found to produce positive responses at 620, 575 and 546 nm band passes in 9 of 10 employees, including employee J.H. Bands 8, 9 and 10 were, however, positive with employees 11 and 12, except band 9 at band pass 575 nm in employee 11, and technical errors in the isolation and preparation of the bands at the start of the investigation may well have been the explanation for the original negative results. It was possible to repeat the photopatch testing procedure with Case 1 in March, 1971, and positive responses were obtained this time to each of the bands 8, 9 and 10 (see Table III).

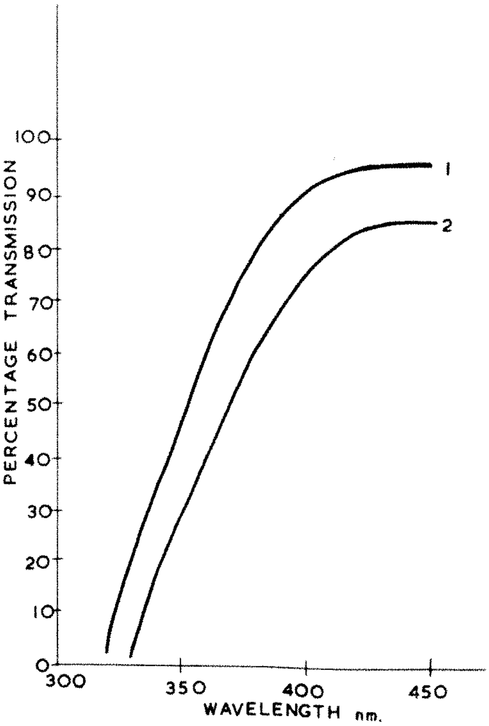

FIG. 2.—Transmission characteristics of Pyrex glass (1) 3/16 in thick, (2) ⅜ in thick

It is interesting that Case 5—W.N., although a control subject from another part of the factory, who had never been in contact with the dye, produced virtually the same results on photopatch testing as did those with a history of intimate contact with the manufacturing process for many years (i.e. positive responses with the immediate precursor and band 4). These findings were subsequently confirmed in 2 of 3 additional control subjects (Cases 15 and 16), who gave positive responses to photopatch testing to the complete dye and the immediate precursor.

In Cases 9 and 10 positive results with the intermediate precursor were not obtained (except at 620 nm in Case 9), and this was probably due to a change of technique, in that the chemical was applied " open " for 20 min prior to irradiation and not, as in the other cases, as a 24-h closed patch test. It was possible in both these subjects, however, to obtain positive responses with the immediate precursor by using a 6 kW xenon light source filtered to allow irradiation with wavelengths above 400 nm (exposure dose 60,000 mJ/cm^2), and, even after a 20-min prior application of the immediate precursor. The evidence therefore suggests that band 4, the immediate precursor, and bands 8, 9 and 10, are responsible for the photocontact reaction.

Due to technical difficulties band 5 was not available for photopatch testing, but its inclusion in the immediate precursor, plus its wavelength peak of 560 nm, would suggest that it possibly also was involved in the photocontact reaction.

Band 1 was excluded from further testing because of its wavelength peak of 428 nm, which was far distant from the previously shown active wavelengths between 500 and 670 nm. Its lack of significance was further supported by the uniformly negative responses with the immediate precursor (contains band 1) when irradiated at 428 nm.

Two employees (Cases 9 and 10) were further studied by applying band 4, the immediate precursor, and the complete dye, 20 min prior to irradiation, using a 6 Kw xenon source with an oven glass (Pyrex) filter with transmission characteristics shown in Fig 2 which transmitted mainly visible light above 400 nm, and it was found that the positive responses were just as easily obtained as those following a 24-h occlusive patch with the monochromator system (Table IV). Thus it was possible to devise a screening method using less sophisticated equipment in the form of a xenon light source and appropriate filters in place of the grating monochromator. The application of this screening method in the factory environment is now being developed.

In no employee was there evidence of photosensitivity in the absence of the chemicals or of allergic contact dermatitis by patch testing in the absence of light.

DISCUSSION

Using the method described, it was possible to show that neither the dye : Disperse Blue 35 nor its 10 constituent bands were causing an abnormal skin reaction as a result of delayed allergic sensitivity, nor was there evidence in any of the employees of photosensitivity to visible light in the absence of the chemicals. It was possible to show, however, that the complete dye : Disperse Blue 35 was capable of producing a photocontact reaction in an individual whether he had been previously exposed to the substance or not, and that only some and not all of its constituent bands were responsible. Despite the occasional unexplained negative or positive response, the sum of evidence favoured bands 4, 8, 9 and 10, and the immediate precursor as being responsible.

The results of this investigation illustrate the relative ease with which photocontact dermatitis in a chemical manufacturing process can be studied, and, even although the product is a complex substance, such as a dye, the investigation can be directed towards determining the responsible constituents and thus the stages of the manufacturing process which require modification or at least improved protection. The initial failure to confirm the responsibility of the completed dye was due to the type of irradiation equipment used, and this underlines the importance of using phototesting equipment which is capable of producing irradiation at selected wavebands.

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SCANNING ELECTRON MICROSCOPY OF THE STRATUM CORNEUM

RODNEY P. R. DAWBER, RONALD MARKS AND J. ALAN SWIFT

Summary.—This report describes studies of stratum corneum from normal subjects and from patients with various scaly disorders, with the scanning electron microscope, using the skin surface biopsy technique.

***Normal* squamous cells showed essentially the same structure in different parts of the body; individual squames demonstrated irregular surface folds, convolutions and occasionally a more villous appearance. In dominant ichthyosis vulgaris the squamous cells showed predominantly an irregular fine nodular surface pattern. Similar changes were found in non-bullous ichthyosiform erythroderma. Surface depressions (maximum diameter approximately 300 nm) were occasionally seen on squames from bullous ichthyosiform erythroderma. Psoriatic squamous cells showed a more pronounced villous pattern than normal squames, the villi being evenly distributed over the surface of the majority of cells examined; some cells had rather flattened villi near their edges.**

Possible physiological roles for the structural changes found are discussed.

We have recently described a technique (skin surface biopsy) which allows detailed examination of squamous cells and which preserves their *in vivo* interrelationships (Marks and Dawber, 1971). Preliminary studies of skin surface biopsies from normal skin, psoriatic plaques and from patients with ichthyosis vulgaris, using the scanning electron microscope, revealed distinct differences in the surface morphology of individual squamous cells.

In this report we describe further studies of stratum corneum from normal subjects, and patients with psoriasis and the ichthyotic disorders, with the scanning electron microscope, using the skin surface biopsy technique, and discuss possible physiological roles for the structural differences found.

MATERIALS AND METHOD

Skin surface biopsies were taken as follows:

A drop of the cyanoacrylate adhesive Permabond (Staident Products Ltd., Staines, Middx., England) was placed on the area of skin under study. A clean glass slide was placed on the drop and pressure applied. The slide was removed after 20–30 sec, taking with it an attached sheet of stratum corneum (3–6 cells thick). Surface biopsies were taken from the flexor and extensor aspects of the forearm, the chest, abdomen and interscapular region in 5 normal subjects, and from clinically involved areas in patients with psoriasis (7 subjects), ichthyosis vulgaris (4 subjects) and both bullous (1 subject) and non-bullous (2 subjects) ichthyosiform erythroderma.

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The glass slides with attached biopsies were cut to the requisite size and mounted on scanning electron microscope " stubs ". Alternatively the biopsies were carefully separated from the slides with a razor blade before mounting. The specimens were coated with a layer of carbon (approximately 50 nm thick) in an Edwards 12 E 6 vacuum coating unit and then transferred to the microscope (Cambridge Stereoscan Mark II) for viewing and photography.

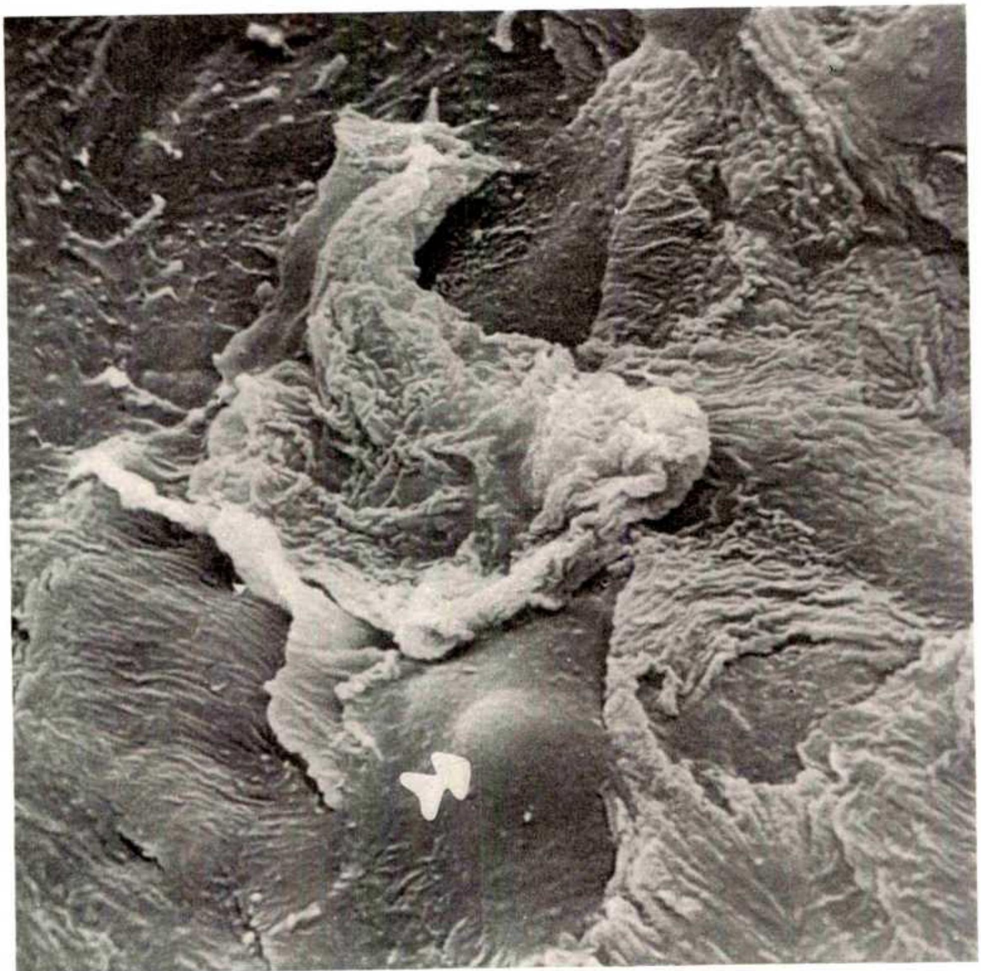

FIG. 1.—*Normal skin.* Individual squamous cells show irregular folds, convolutions and villi. The bulge (white arrow) is thought to represent (at the cell surface) the position of the nucleus before cell death (× 2000).

RESULTS

Normal skin.—Surface biopsies from the different sites examined all showed basically the same structural pattern. At magnifications of less than × 100 the epidermal furrows were visible. The surface morphology of individual squamous cells was clearly visible at magnifications of greater than × 500. All cells showed irregular folds, convolutions and occasionally a more pronounced villous appearance

(Fig. 1). Cell margins were often difficult to see, adjacent cells appearing to merge because of their close apposition. Consequently, desmosomal contacts were only occasionally visible at the cell margins. A central bulge or depression (15–20 μ diameter) was present on a few cells, thought to be related to the position of the nucleus before " cell death " (Fig. 1). Mean cell size (horizontal diameter across the centre of the cell) in surface biopsies from normal skin was 45 μ.

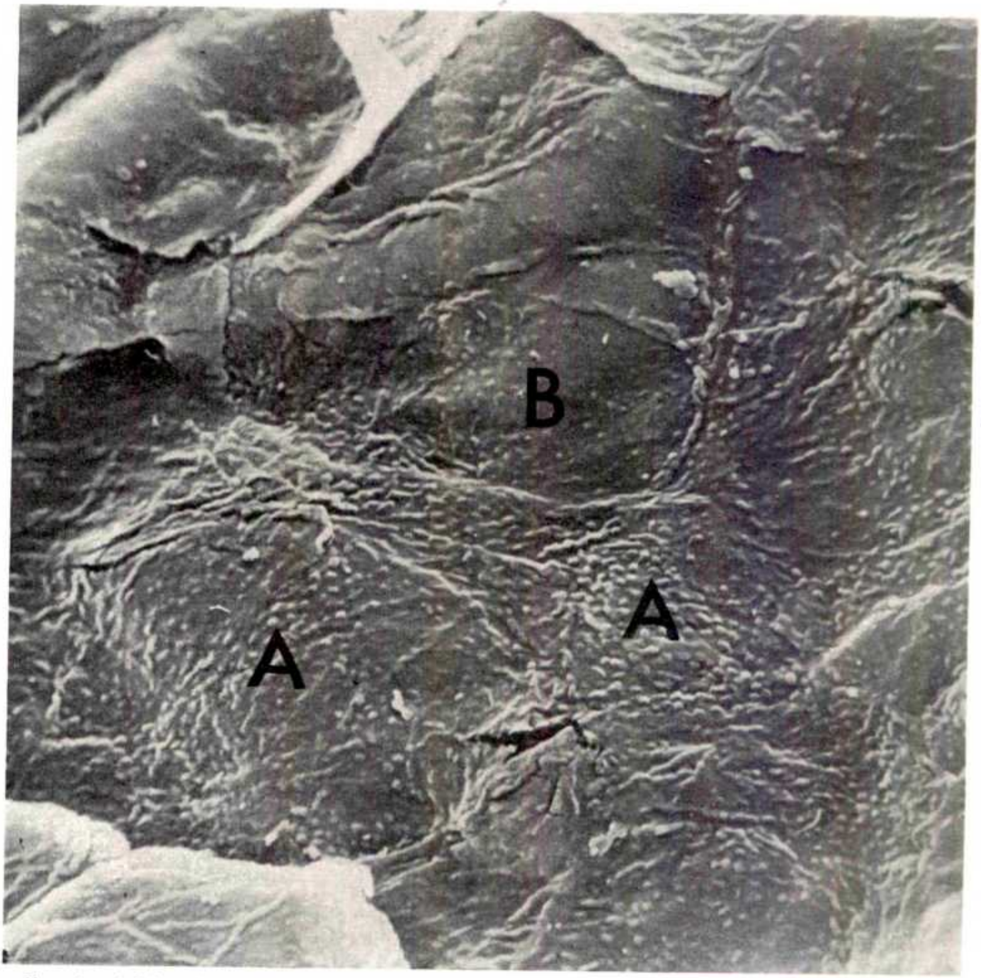

FIG. 2.—*Ichthyosis vulgaris.* Characteristic squames with a fine nodular surface appearance (A) and 1 cell having a predominantly flat surface (B) (× 2000).

Dominant ichthyosis vulgaris.—The epidermal pattern at low magnifications (× 100) appeared normal. The predominant structure on individual squames was an irregular fine nodular pattern, present in approximately 80% of the cells examined; the remaining cells were flat, showing only occasional small nodules (Fig. 2). The 2 cell types were quite randomly arranged and the 4 : 1 ratio of nodular to flat cells was essentially the same in all the biopsies examined. Rarely a cell was seen with a convoluted surface, as seen in normal skin. Mean cell diameter (42 μ) was not significantly different from that found in normal skin.

Bullous and non-bullous ichthyosiform erythroderma.—The findings in these 2 conditions were essentially similar. Virtually all the cells examined had a flat surface appearance (Fig. 3, 4). In the non-bullous form a few cells possessed fine irregular nodular structures similar to those seen in ichthyosis vulgaris (Fig. 5); in contrast to normal skin, cell margins were usually clearly visible, as were the desmosomal attachments between adjoining squamous cells (Fig. 5). In the bullous form, surface depressions (maximum diameter approximately 300 nm) were occasionally seen (Fig. 4); 1 surface biopsy taken from a non-bullous area removed the whole epidermis, rete pegs, sweat ducts and hair follicles being visible at the dermo-epidermal junction. Mean cell size in bullous ichthyosiform erythroderma

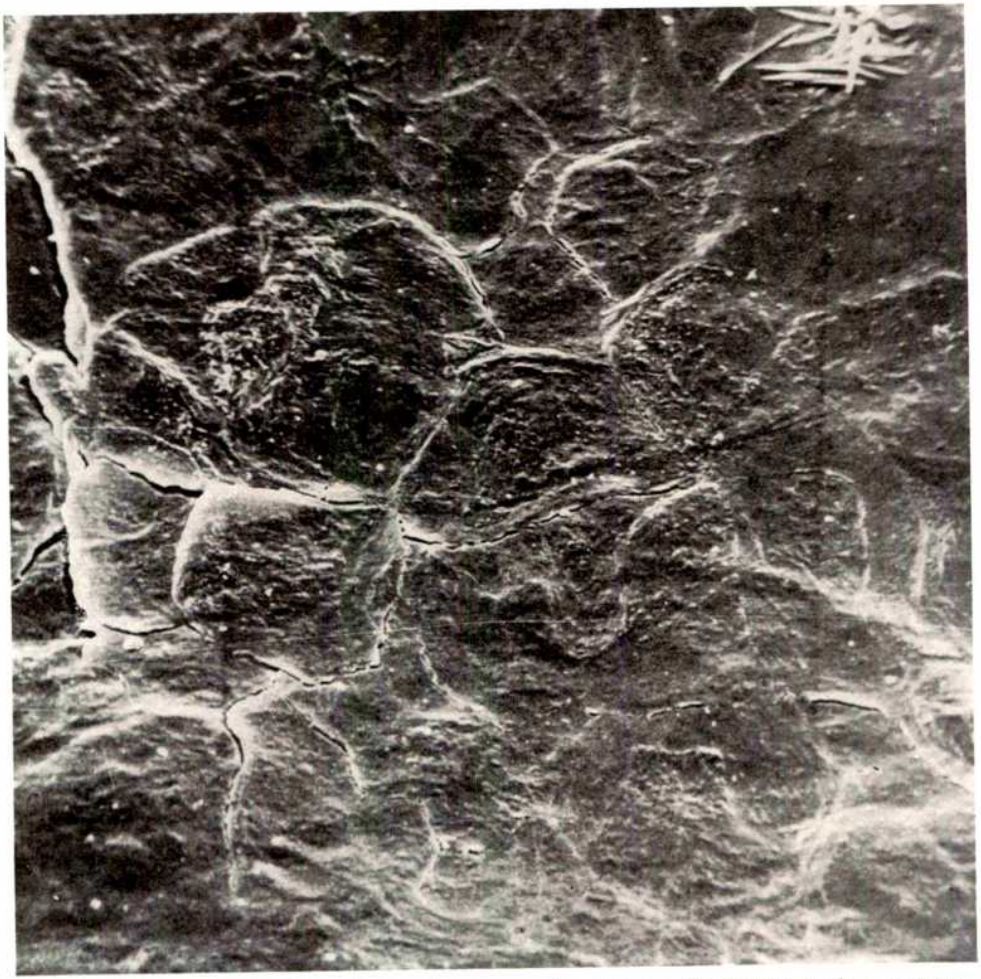

FIG. 3.—*Non-bullous ichthyosiform erythroderma.* Squamous cells show a surface pattern flatter than that of normal skin; the edge of most cells is easily visible (× 1000).

(49 μ) was greater than in the non-bullous form (44 μ), but this difference was not significant.

Psoriasis.—Surface biopsies from typical psoriatic plaques showed absence of the epidermal furrows that were seen prominently in normal skin and ichthyosis vulgaris at magnifications below × 100. A large majority of squamous cells revealed a pronounced villous pattern in all biopsies (Fig. 6, 7). The villi (diameter approximately 350 nm) occurred evenly over the cell surface, apart from a narrow area around the periphery, at which the villi were either flattened or absent (Fig. 6). Occasional cells showed surface depressions of similar size to the villi (Fig. 8). On most biopsies, grooves were seen (Fig. 8), their hexagonal pattern

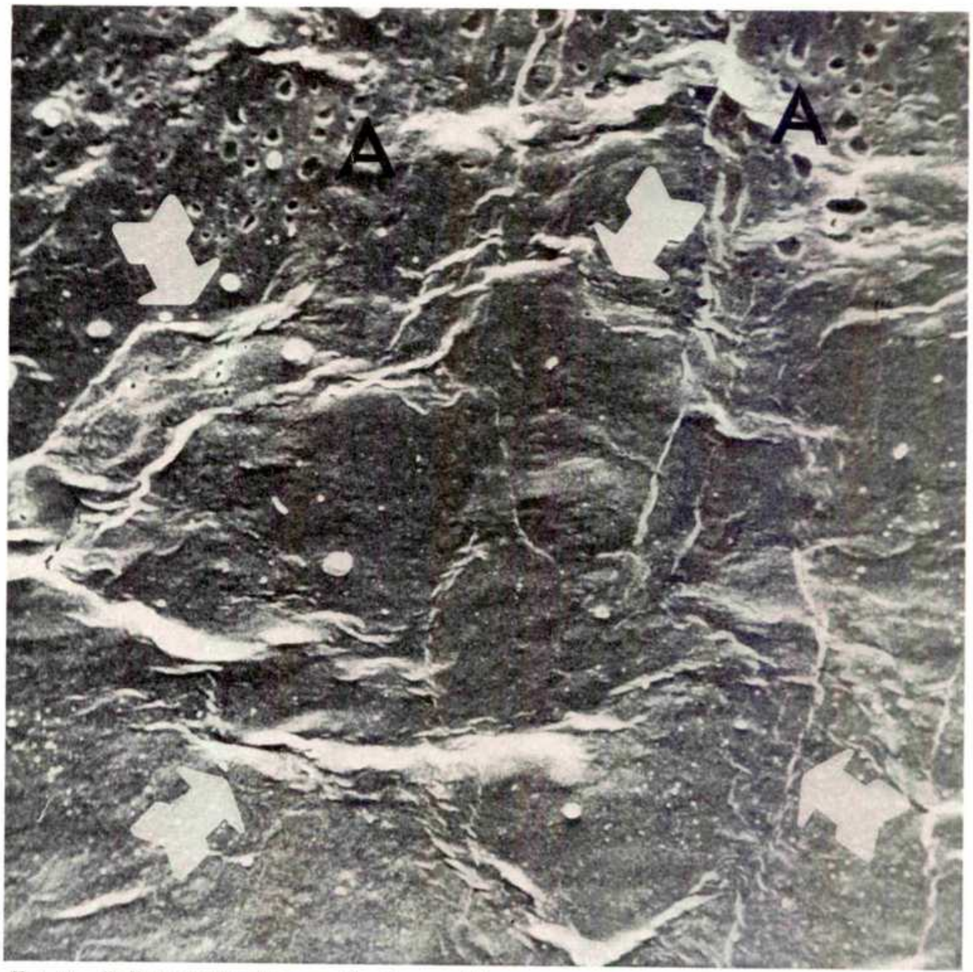

FIG. 4.—*Bullous ichthyosiform erythroderma.* The squame seen centrally (bounded by white arrows) has a relatively flat surface. Several other cells possess surface depressions of pits (A) (× 2000).

suggesting that they represent the outline of squamous cells in the next layer that *in vivo* were closely apposed to the layer viewed here.

DISCUSSION

Examination of skin sections with the conventional transmission electron microscope gives little insight into the 3-dimensional arrangement of the cornified cells, and involves the use of fixation processes which may give rise to artefacts. No prior fixation is required for scanning electron microscopy of skin surface biopsies; in addition, the horizontal plane can be satisfactorily inspected, the final image having a 3-dimensional appearance.

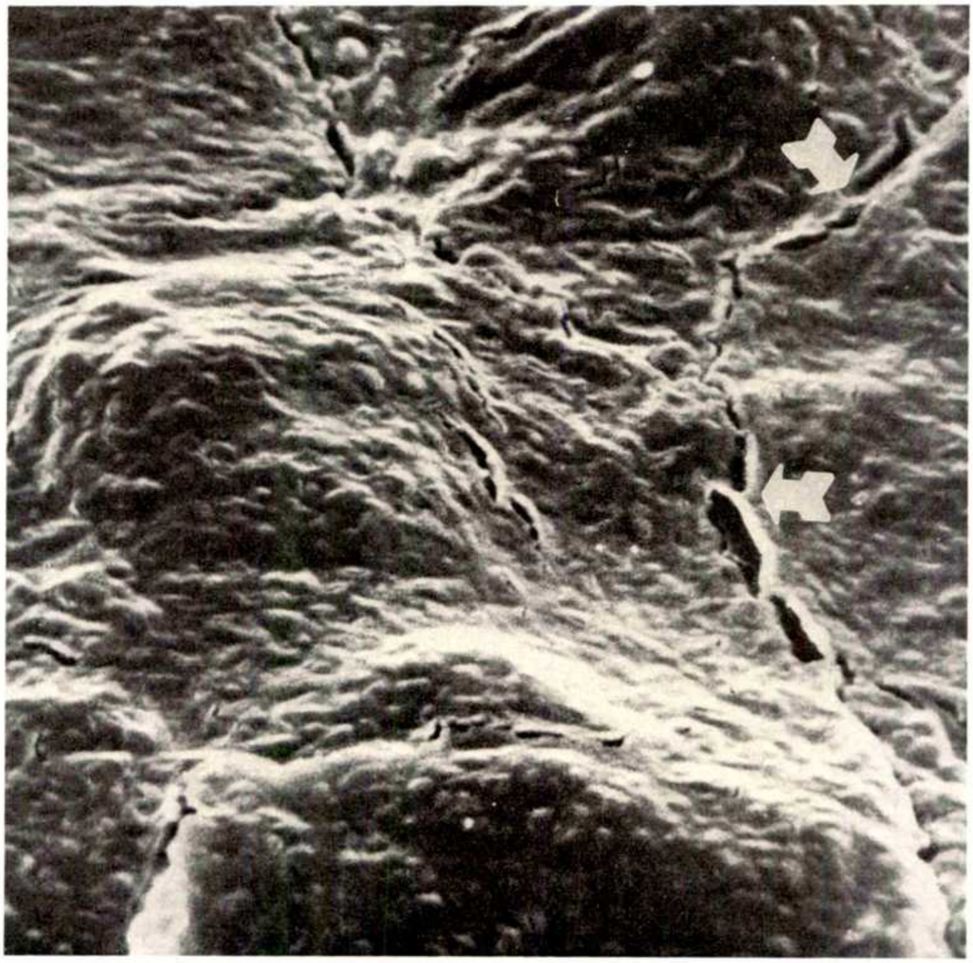

FIG. 5.—*Non-bullous ichthyosiform erythroderma.* Fine nodular appearance of cells, with desmosomes (white arrows) visible between them (× 5000).

The undulations and villi on the surface of individual squames may represent the site of desmosomes, since they occur with the same frequency and are of similar size to desmosomes seen with the transmission electron microscope.

The above observations in normal and psoriatic stratum corneum confirm our preliminary findings and are broadly in agreement with those of Mishima (1971). In the latter study the surface depressions found in psoriasis were termed " pores ". The term " depressions " is preferable, since transmission electron microscopy has not demonstrated pores (Brody, 1962). They are therefore likely to be depressions in the plasma membranes rather than perforations. Mishima (1971) postulated that these " pores " could contribute to the increased rate of transport of materials through psoriatic epidermis (Scott, 1959; Malkinson, 1958). The prominent surface villi and depressions of squamous cells could contribute to more rapid transfer of substances through the stratum corneum because of the greater area available at the cell surface.

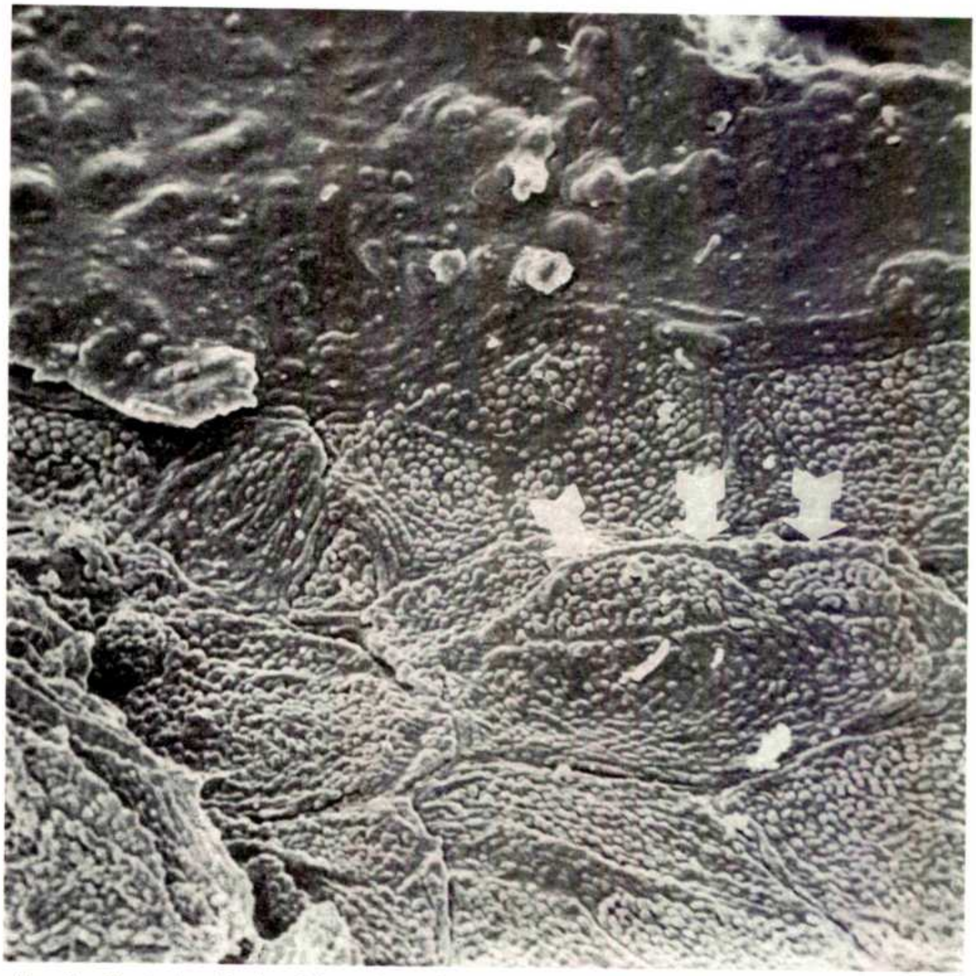

FIG. 6.—*Psoriasis*. Surface biopsy from the edge of a psoriatic plaque. The upper third of the figure shows normal cells, whilst the lower two-thirds shows psoriatic squames with prominent surface villi. Some of the latter have a flattened margin (white arrows) (× 2000).

Of the conditions studied here, psoriasis has shown the most prominent change. Since the characteristic abnormality in psoriatic stratum corneum is parakeratosis, the villous pattern seen in this study may be due to the presence of parakeratotic cells. In support of this is the fact that prominent villous structures have also been seen on dandruff scales and on scales from the earlobe in seborrhoeic dermatitis, both of which are parakeratotic conditions (Swift, 1971, personal observations).

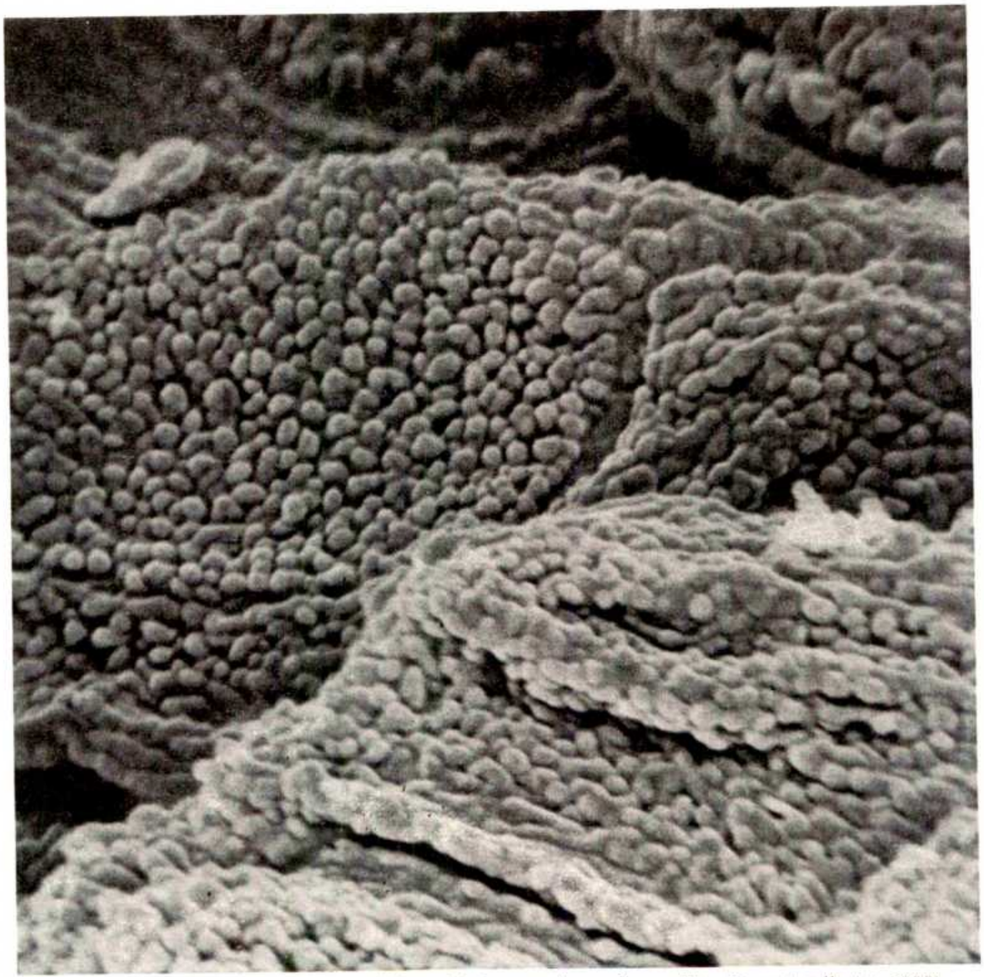

FIG. 7.—*Psoriasis.* Prominent villi studded over the surface of 3 adjacent cells (× 5000).

Studies of the cells in the granular layer in normal epidermis have shown prominent surface interdigitating folds, which become less obvious in the stratum corneum. Consequently the villi seen on psoriatic squames in this study may be a reflection of the normal granular layer, since parakeratotic cells are only " recent arrivals " in the stratum corneum.

McKenzie (1969) has shown that, in normal epidermis, squamous cells are arranged in vertical columns. These columns are found in all parts of the body except the palms and soles, where the squames appear to be randomly arranged (Menton and Eisen, 1971). Psoriatic stratum corneum would appear to be similar to the palm and sole in this respect, since the hexagonal groove pattern made by the next layer of squames would not be present if cells were arranged in vertical columns in psoriasis. Grooves were not seen in the other disorders studied here.

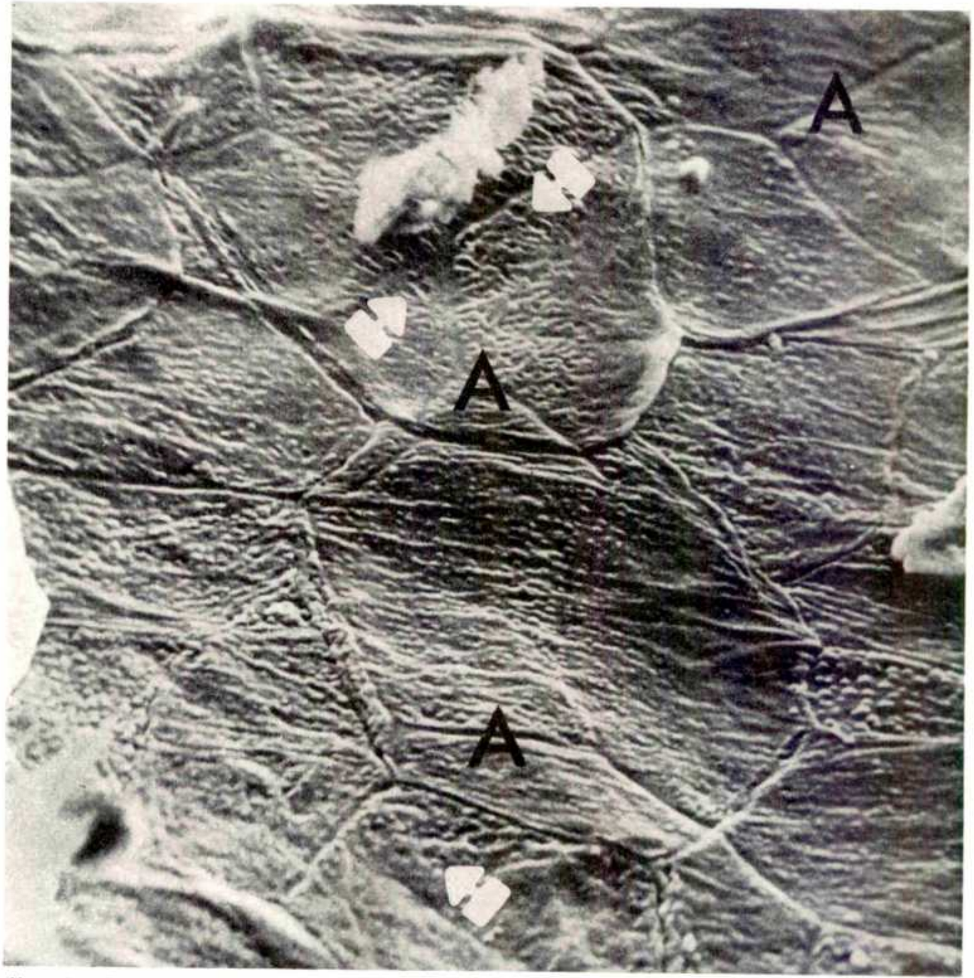

Fig. 8.—*Psoriasis.* Most cells have rather flatter nodules and villi here; a few surface depressions are seen irregularly (white arrows). A series of grooves (A) is visible, which cross cell margins, forming hexagonal patterns (× 2000).

It has been emphasized that a prominent feature of psoriasis is the cohesiveness of the scales (Flesch and Esoda, 1958), whereas in ichthyosis the reverse would appear to be the case. Our observations could in part explain this; if the villi and depressions of psoriatic squames interdigitate, as appears to be the case from conventional transmission electron microscopic observations, they will be in more intimate contact and have greater stability and adhesiveness than cells with a relatively flat surface, even without alteration in the adhesiveness of the intercellular cement. This principle has been postulated as an important stabilizing mechanism in the normal epidermis (Montagna and Lobitz, 1964).

Because this technique enables the surface of the stratum corneum to be examined in a relatively undistorted condition, it adds a new dimension to the study of this layer. Further studies are at present in progress to investigate the effect of treatment on these scaly dermatoses and to cover other diseases involving the stratum corneum.

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THE RESPONSE OF THE SWEAT GLANDS TO β-ADRENERGIC STIMULATION

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Summary.—Intracutaneous injections of isoprenaline gave a sweat response in 8 of 13 subjects. Injections of isoprenaline, together with an α-blocking agent, gave equal or higher sweat responses. The curves obtained suggested that the responses were mediated by acetylcholine.

It is suggested that some clinical observations might reveal whether a β-adrenergic mechanism is possibly involved in the paradoxical sweat reactions of patients in shock, in hypoglycaemic patients and in patients with a phaeochromocytoma.

THE cholinergically innervated sweat glands may also respond to intracutaneous injections of adrenaline. The minimal effective concentration was reported to be 10^{-5} mg/0·1 ml (Wada, 1950; Chalmers and Keele, 1951). Concentrations of 10^{-3} to 10^{-2} mg/0·1 ml were found to be equally effective in producing a maximal response (Warndorff and Neefs, 1971).

The mechanism and the physiological significance of the sweat responses to adrenaline have not been clarified. Adrenaline possesses both α- and β-mimetic activity. Although sweat responses to intracutaneous injections of the synthetic β-mimetic agent isopropylnoradrenaline (Isuprel, isoprenaline) have been reported (Haimovici, 1950; Sonnenschein *et al.*, 1951), the sweat responses to adrenaline are usually considered to depend upon its α-mimetic activity, since they may be inhibited by α-blocking agents and because responses similar to those from adrenaline occur with noradrenaline, which has mainly α-mimetic activity (Sonnenschein *et al.*, 1951; Foster and Weiner, 1970). In explanation of the sweat responses to the β-mimetic isopropylnoradrenaline it has been suggested that it is converted into noradrenaline in the skin (Haimovici, 1950). This suggestion has not been checked by testing the influence of α-blocking agents on these responses.

Using a technique for continuous recording of sweat gland activity (van Gasselt and Vierhout, 1963; Warndorff, 1970) we have compared the sweat responses to isoprenaline with those obtained in experiments with adrenaline (Warndorff and Neefs, 1971) and measured the influence of α-blocking agents on the isoprenaline-responses.

MATERIALS AND METHODS

The subjects were 5 healthy volunteers (4 males, 1 female) and 10 dermatological outpatients (5 males, 5 females). Their ages were between 19 and 42 years. Among the patients were 6 with minor local ailments, 3 with atopic dermatitis and 1 with lichen planus.

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The investigations were carried out, between January and July, on the volar aspect of the forearm, at room temperatures between 21°C and 24°C. The subjects were comfortably seated, relaxed and adapted to the environment. A cup of 0·2 cm² was applied to the skin and held in place by Elastoplast strips over the connecting tubes, using the minimum pressure sufficient to avoid leakage of the carrier gas.

All injections were made below the centre of the measuring cup in a volume of approximately 0·1 ml. Different tests were always carried out at different sites. Sweat gland activity was measured by means of the electrolytic water analyser "Meeco", as described by van Gasselt and Vierhout (1963).

EXPERIMENTAL

In 13 subjects the sweat glands were stimulated with 10^{-3} mg isoprenaline. In 12 of these subjects a test was also carried out with 10^{-3} mg isoprenaline together with 10^{-3} mg Rogitine, or together with 10^{-2} mg piperoxan only. In the remaining 2 subjects the response to this mixture was observed. Thus the total number of tests was 27.

From the responses obtained the sweat rates were recorded and compared.

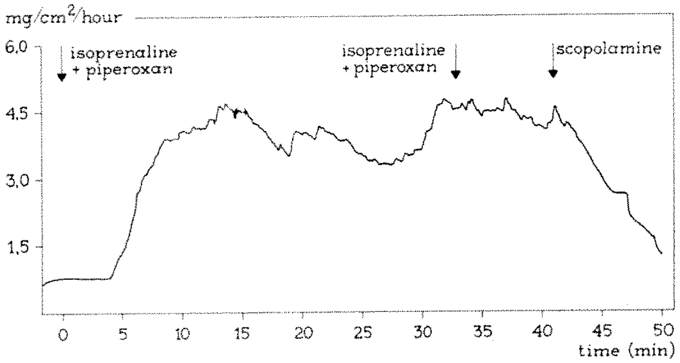

FIG. 1.—The response curve of one of the subjects to 2 intracutaneous injections of 10^{-3} mg isoprenaline + 10^{-2} mg piperoxan. The sweat response was terminated with 10^{-3} mg scopolamine.

RESULTS

Positive sweat responses were obtained in 8 of the 13 tests with isoprenaline and in 9 of the 14 tests with isoprenaline + α-blocking agent.

From the 12 subjects tested with both solutions, 4 showed the greatest response to isoprenaline + α-blocking agent, 3 showed about equal responses and 4 responded to none; 1 subject did not respond to isoprenaline, but showed a small response to isoprenaline + α-blocking agent.

The latency between the moment of the injection and the onset of the response varied from 2 to 8 min.

The most surprising finding was that the curves obtained resembled those of low-grade spontaneous sweating. Similar curves, but usually with higher sweat rates, characterized by spikes of variable amplitude and by fluctuations in the sweat responses, were obtained in this laboratory after stimulation of the sweat glands by physical exercise or by thermal stimulation in the dry heat sauna.

In 14 tests the maximal sweat rates varied between 1·5 and 6 mg.cm^{-2}/h. In the other 3 tests they varied between 9 and 15 mg.cm^{-2}/h.

Six sweat responses with maximal sweat rates below 2 mg.cm^{-2}/h terminated spontaneously after 5 to 12 min. The other 11 responses did not, and their course was followed for a period of 15 to 37 min. In 5 subjects an intracutaneous injection of 10^{-2} mg atropine or 10^{-3} mg scopolamine in 0·1 ml was given during the response, and this appeared to terminate the sweat reaction (Fig. 1). No differences were observed between the responses of the males and of the females.

DISCUSSION

The curves obtained in the sweat responses to isoprenaline were clearly different from those obtained by local injections of adrenaline or noradrenaline (Foster *et al.*, 1970; Warndorff and Neefs, 1971). The α-blocking agents did not inhibit the responses, but on the contrary seemed to augment them. It is therefore unlikely that the injected isoprenaline was converted into noradrenaline in the skin as was suggested by Haimovici (1950). The similarity of the curves to those of spontaneous sweat reactions and the effect of a specific anticholinergic agent suggests that the sweat responses were caused by acetylcholine.

During the experiments the subjects were relaxed and adapted to the environment; and, from the straight line of the recorder, it appeared that they were not spontaneously sweating before the injections were given. It seems that isoprenaline caused a periodic release of acetylcholine or an increase in the sensitivity of the secretory cells to the little amounts of acetylcholine which normally leak from the nerve endings (Emmelin, 1965); possibly isoprenaline interfered with the breakdown of acetylcholine.

Up to now we have not succeeded in finding the experimental conditions or circumstances under which similar responses can be obtained to adrenaline. Such conditions or circumstances might well exist, and their discovery could possibly shed light on some serious contradictory results in the literature concerning the duration of the sweat responses to adrenaline and the effects of α-blocking agents and of atropine on these responses (Wada, 1950; Dutta, 1967; Sonnenschein *et al.*, 1951). However, even if a β-mimetic effect of adrenaline on the sweat glands could be demonstrated, the question would remain as to whether this is a nonspecific effect or somehow related to the normal function of the sweat glands.

Some clinical observations on the sweat reactions of patients in shock, hypoglycaemic patients and patients with a phaeochromocytoma might contribute to the solution of this problem. These sweat reactions paradoxically co-exist with an extreme vasoconstriction due to strong sympathetic impulses and a rise in the level of serum-catecholamines up to 100 times the normal value (Lund, 1952; Zierott, 1971).

It has been demonstrated that the sweat reactions are not caused by α-adrenergic stimulation of the sweat glands but that they are mediated by acetylcholine (Chalmers and Keele, 1952; Prout and Wardell, 1969). The question is whether or not this implies a nervous stimulation. Under experimental circumstances spontaneous sweat reactions have been shown to be strongly inhibited by intra-arterial injections of adrenaline in concentrations comparable to those which may exist in the pathological conditions mentioned (Foster *et al.*, 1970), and also by local injections of adrenaline (Elliot, 1905; Kuno, 1956; Warndorff, 1968, unpublished observation). Possibly the sweat reactions in the above-mentioned patients are related to the β-mimetic effect of the increased amount of circulating adrenaline, in which case they could be inhibited by β-blocking agents. Some cardiac patients are nowadays treated with β-blocking agents. They would form a " natural experiment " if their sweat responses were observed in case of shock.

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A NEW ULTRASTRUCTURAL FINDING IN ORAL SUBMUCOUS FIBROSIS

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Summary.—Oral submucous fibrosis and progressive systemic sclerosis (scleroderma) show clinical and histological similarities, but there are also differences in distribution and prognosis. So far there has been no evidence of any connection between these diseases.

Transmission electron microscopy of connective tissue from a patient with oral submucous fibrosis shows, however, that the normal pattern of uniform size fibrils gathered together in bundles is replaced by an excess of fine (immature) fibrils and of interfibrillar matrix. The average fibril diameter was 36·2 nm compared with the normal of 49·7 nm from the same area, while the concentration was 210 to 240 fibrils/μm^2 compared with 170 to 180 in the normal. Though the ultimate causes of progressive systemic sclerosis and of oral submucous fibrosis probably differ, it seems likely that they share a defect of collagen maturation which gives rise to a clinically similar lesion.

ORAL submucous fibrosis (OSMF) is a slowly progressive disease of the oral cavity and occasionally pharynx and oesophagus, affecting Indians almost exclusively. The main feature is subepithelial fibrosis and mucosal rigidity, causing limitation of opening the mouth or immobility of the soft palate. Epidemiological, clinical and histological aspects of the disease have been described by Pindborg and his co-workers (Pindborg and Sirsat, 1966; Pindborg *et al.*, 1968; Hamner *et al.*, 1971), and there is some evidence (Pindborg *et al.*, 1967) that it may be a factor contributing to the high incidence of oral cancer in India. The cause is unknown, but although OSMF has clinical and histological features in common with scleroderma, the distribution of the lesions is obviously different and there has been so far no evidence to link the diseases together. A new finding in the ultrastructure of the fibrous tissue in OSMF relevant to this problem is reported here.

MATERIAL AND METHODS

A biopsy was taken from the buccal mucosa of a moderately severely affected Indian man aged 32 who had opening of the mouth limited to 2·5 cm. The specimen was fixed in cold 4% paraformaldehyde, hemisected, and half was embedded in paraffin for routine processing. The remainder was prepared for electron microscopy by staining in osmium tetroxide for 2 hours, dehydrating and embedding in Taab resin. Sections were cut on an LKB ultratome. Alternate sections were cut at 2 μm thickness, stained with Paragon (Paragon C and C Co. Inc., N.Y.) and examined with a light microscope to orientate the material.

In addition another specimen was taken from the same area of buccal mucosa of a

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healthy British male of the same age and processed as described above for direct comparison. Both specimens were examined with an RCA EMU4 electron microscope, and fibrils were measured on photographic prints at ×65,000.

RESULTS

The characteristic feature of OSMF under the light microscope is a homogeneous, collagenous, subepithelial zone of irregular depth, with little bundle formation, but in which degenerating muscle fibres can be seen. In this specimen there were no obvious differences in staining reaction of ground substance between OSMF and normal buccal mucosa with the periodic acid-Schiff reaction or alcian blue. OSMF connective tissue was, however, found to have a higher content of (a) argyrophil fibres when stained by Gordon and Sweet's method for reticulin, (b) elastin fibres (LEHVG stain, Postgraduate Medical School, Technical Methods Book (1961)), and (c) oxytalan fibres (Fullmer and Lillie, 1958).

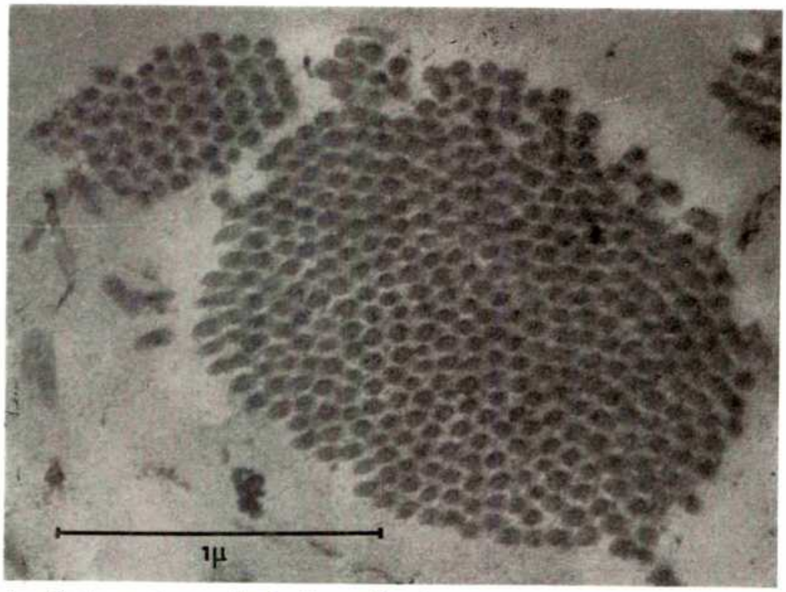

FIG. 1.—Electron micrograph of collagen fibre from normal buccal mucosa. The fibrils are of regular diameter and closely packed. (×65,000.)

The main ultrastructural features of normal connective tissue are fibrils which are of uniform size (Harkness, 1964) arranged compactly in bundles (fibres); these fibres are relatively well spaced, lying in a matrix of ground substance (Fig. 1). In all the subsequent measurements on normal connective tissue it should be noted that no account has been taken of the area of interfibre matrix, all measurements being taken within individual fibres. This orderly arrangement of fibres and matrix was not seen in OSMF; and, on the basis of measurements on 1190 fibrils in the 2 specimens, the 3 main findings in OSMF were as follows (Fig. 2):

1. There was no true bundle (fibre) formation in OSMF. The fibrils (which in the normal tissue form the fibres) were dispersed throughout the ground substance with varying but relatively wide intervals between them. In the areas of highest

concentration 210 to 240 fibrils/μm^2 were present. Fibres in normal mucosa showed 170 to 180 fibrils/μm^2.

2. In OSMF the fibrils showed a much wider variation in diameter than the normal, but overall were significantly thinner than normal. The average diameter, on the photographic print, of 531 fibrils from fibres in normal mucosa was 3·23 mm (actual size of fibril approximately 49·7 nm), with 95% measuring between 2·30 mm (35·5 nm) and 3·85 mm (59 nm). The average diameter of 659 fibrils in the denser areas of OSMF was 2·35 mm (36·2 nm), with 95% measuring between 1·10 mm (17 nm) and 3·45 mm (53 nm) (Fig. 3).

3. There was more interfibrillar " space " per unit area in OSMF, even in the areas of greatest fibril concentration, than in normal buccal submucosa.

There was, however, no difference in the banding interval in either specimen, both being 64–70 nm.

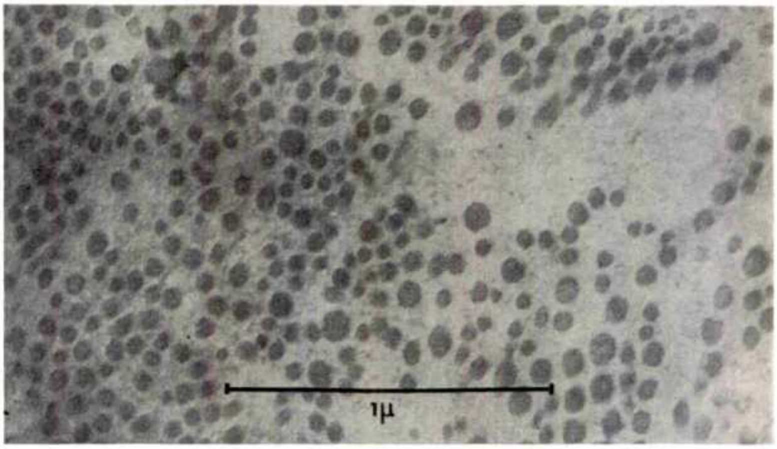

FIG. 2.—Electron micrograph of collagen fibrils in OSMF. They have a random distribution and show great variation in diameter. (× 65,000.)

It appears, therefore, that, in OSMF, the fibrils are thinner and not arranged in compact bundles to form fibres as in normal connective tissue but irregular in size and irregularly distributed throughout the ground substance. Although the individual fibrils in OSMF are more widely spaced than in normal connective tissue, they are overall of so much smaller diameter that the average number of fibrils in unit area is greater than in the normal fibre, even when no account is taken of the area of ground substance between the fibres of normal connective tissue.

DISCUSSION

Oral submucous fibrosis and scleroderma apparently, therefore, share the ultrastructural feature of a predominance of thinner fibrils, as shown by Braun-Falco and Rupec (1964) and Hayes and Rodnan (1971). The latter found the fibril diameters had a peak value in the range of 30–50 nm, i.e. close to that reported here. On the other hand, it does not appear from Braun-Falco and Rupec's report that the fibrils are so widely dispersed in scleroderma, and their micrograph indicates fibril spacing similar to that in normal connective tissue.

The finding in OSMF of overall thinner fibrils than in normal connective tissue suggests that, as in scleroderma (Braun-Falco and Rupec, 1964), the fibrils are embryonic in type and that the defect lies in polymerization and maturation. There is excess production of young collagen fibrils per unit area and this is further supported by the increased argyrophilia in tissue sections examined by light microscopy.

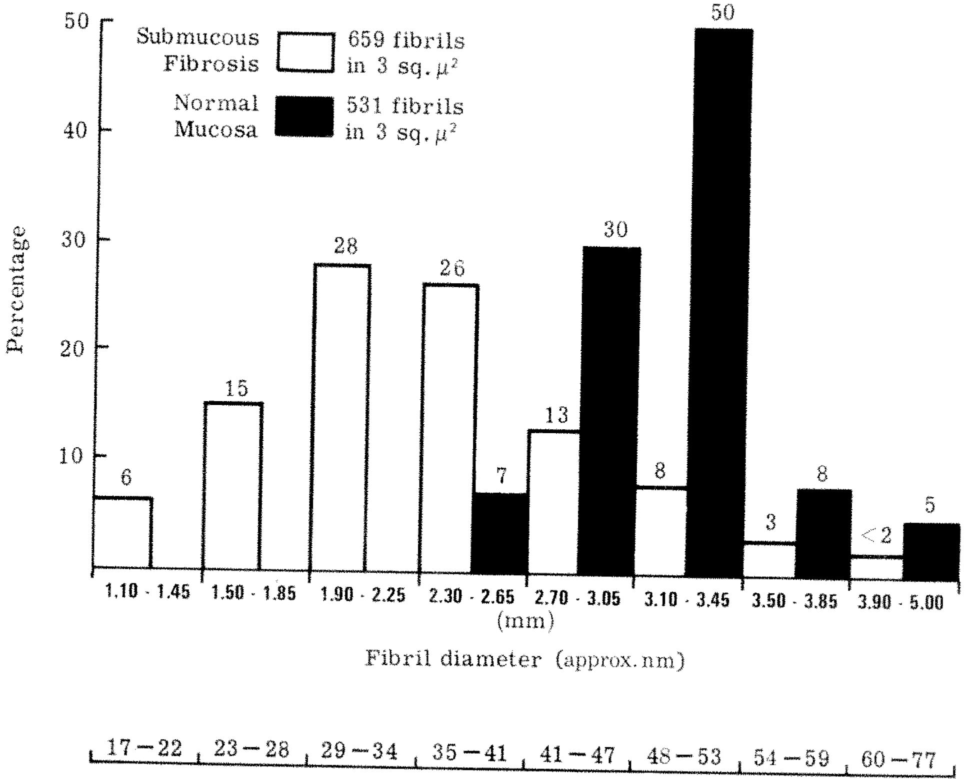

FIG. 3.—Percentage distribution of collagen fibrils of varying thickness, in OSMF and normal buccal mucosa.

We are grateful to Mr K. C. Rajasingham for preparing this material and taking the electron micrographs.

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OUT-PATIENT TREATMENT OF ICHTHYOSIS: A DOUBLE-BLIND TRIAL OF OINTMENTS

F. M. POPE, J. K. REES, R. S. WELLS AND K. G. S. LEWIS

Summary.—Eighty-four out-patients with either ichthyosis vulgaris or X-linked ichthyosis were treated with 4 topical applications (oily cream, B.P.; salicylic acid ointment, B.P.; a urea cream; and Boots E.45 cream) in a double-blind control trial. At the end of the 2-week treatment period the response was assessed by soliciting the patient's opinion and by personal assessment of the clinicians in charge. The patient's assessment did not reveal any statistically significant difference between the groups, but on the assessment of the clinicians, the urea cream (Calmurid) was statistically significantly better in controlling the ichthyosis than the other 3 preparations.

THE word ichthyosis describes individuals who, in the untreated state, have visible removable cutaneous scales. In all but a few people this disorder is inherited. The most satisfactory initial classification is one based on the mode of inheritance; and, if all types are combined, the minimum incidence in an English population is of the order of 1 in 1000 (Wells and Kerr, 1966). The most commonly seen are ichthyosis vulgaris, inherited as an autosomal dominant trait, and X-linked ichthyosis, which, as the name implies, occurs only in males and is transmitted by clinically unaffected females. The former condition often improves with advancing years, but the latter does not.

Treatment is unsatisfactory (Wells, 1966), because no therapy has been found which produces a permanent improvement. Most patients do not apply treatment of any kind, and pay little regard to the condition. The disability is entirely cosmetic, but may lead to a great deal of anxiety in some individuals. Topical applications may temporarily clear the scales, particularly if applied regularly, and after the skin has been hydrated.

For such a common condition there are very few reports of double-blind trials using reasonably large numbers of patients. Therefore a trial of treatment was undertaken with the co-operation of patients previously seen by Wells and Jennings (1967).

DESIGN OF TRIAL

The trial was concerned primarily with treatments which patients could undertake at home, with little interference to their normal activities. At the same time quantitative assessment of the effect of the treatment was required, because it is insufficient for a new treatment to achieve results only similar in efficacy to existing forms. Cost, side effects, and the willingness and ability of the patient to undertake the treatment must all be

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taken into consideration. Four treatments were used, of which 2 were standard national formulary preparations and 2 commercial products. These are listed below:

1. Oily cream (ung. aquosum, B.P., 1968). This consists of equal quantities of wool alcohol ointment and purified water. It is a water immiscible cream base.
2. Salicylic acid ointment (ung. acid salicyl., B.P., 1968). This consists of 2% salicylic acid in a wool alcohol ointment, B.P. It is used as a keratolytic agent.
3. Calmurid cream (Pharmacia). Calmurid is a cream containing 10% urea in a stabilizing emulsified base. The preparation was used in the treatment of ichthyosis by Swanbeck (1968).
4. E.45 cream (Boots). This consists of white soft paraffin, light liquid paraffin and anhydrous lanolin in a specially formulated emulsion base. This preparation has a general use as a " moisturizing " cream, and was used in the treatment of ichthyosis by Vickers and Sneddon (1961).

Sample

With the approval of their general practitioners, patients were asked by letter if they would like to take part in a trial of treatment. A total of 92 accepted; most were not attending hospital but had either ichthyosis vulgaris (autosomal dominant) or X-linked ichthyosis. They were divided into 4 equal groups of 23 patients. All were asked to stop any former treatment for at least 1 week before the definitive treatment began. Individuals known to have atopic eczema, in addition to ichthyosis vulgaris, and the autosomal recessive types of ichthyosis, were not included in the trial.

METHOD

On their first visit, the patients were graded as mild, moderate or severe, depending upon the number of limb surfaces affected with ichthyosis. Each limb was taken to consist of 4 surfaces (upper: lower, and anterior: posterior) making a total of 8 for the arms and legs. If 7 or 8 surfaces were affected, they were said to be severe; 5 or 6 moderate; and 4 or less, mild. In practice this has been found to give a good indication of the general severity of the ichthyosis on the trunk as well as the limbs (Wells and Jennings, 1967), but for the purposes of this trial some patients were designated as very severe if all surfaces were affected, and if the lesions were particularly thick.

All the patients were given type written instructions so that treatment was applied as uniformly as possible. Each person was given 1 ointment, and their untreated limbs acted as a control.

The instructions were as follows: the ointment given to them was to be applied twice daily to the affected area on 1 leg and 1 arm. Left-handed patients were to apply the preparation to the right arm and leg, and right-handed patients to the left arm and leg. This was to be done after washing or bathing and they were instructed to leave the application on the skin for 3 to 5 min before wiping off the excess.

They were then referred to the pharmacy where they were supplied with 100 g of 1 of the 4 preparations on a random basis.

At the end of a 2-week period of treatment the patients returned for assessment of the response to treatment. This was graded as worse, no change, slight improvement or marked improvement. The patients' own opinions on the response to treatment were recorded as well as those of the examining clinicians (F.M.P., J.R. and R.S.W.). The patients were seen by all 3 clinicians prior to therapy and at the end of the treatment period, when the clinicians were still unaware of the precise treatment each patient had received.

RESULTS

Of the 92 patients who agreed to take part in the study, 8 failed to return for a second visit. They were therefore excluded from the statistical analysis of the therapeutic response. These 8 patients were divided between the 4 groups as follows: 2 from the group receiving oily cream, 1 from the salicylic acid group, 1 from the urea group, and 4 from the E.45 group.

Two patients in the group receiving the oily cream became worse, and 1 of these was forced to stop treatment because of erythema and pruritus.

There were 24 males and 13 females with ichthyosis vulgaris in the study, and 47 males with X-linked ichthyosis (Table I).

While ichthyosis vulgaris is the more common form of this condition, the fact that there were more X-linked ichthyotics in this study was probably because the X-linked type is usually the more severe, and such patients may be more likely to seek medical advice. Since the original ascertainment carried out by Wells and Jennings (1967) was for patients with X-linked ichthyosis, there were more of these available for the study.

TABLE I.—*Distribution of Patients in Each Group According to Sex, Type of Ichthyosis and Severity of the Condition*

		Males		Females
Preparation used	Severity	Ichthyosis vulgaris	X-linked ichthyosis	Ichthyosis vulgaris
Oily cream (B.P. 1968)	Mild	—	—	—
	Moderate	—	1	1
	Severe	2	2	1
	Very severe	5	9	—
Totals		7	12	2
Salicylic acid ointment (B.P., 1968)	Mild	—	—	—
	Moderate	2	1	2
	Severe	4	2	2
	Very severe	1	8	—
Totals		7	11	4
Urea cream (Pharmacia)	Mild	—	—	—
	Moderate	—	1	—
	Severe	4	3	1
	Very severe	2	10	1
Totals		6	14	2
E.45 cream (Boots)	Mild	—	—	—
	Moderate	1	1	1
	Severe	3	3	3
	Very severe	—	6	1
Totals		4	10	5
Grand total		24	47	13

Severity

The data on the varying degrees of severity of the X-linked ichthyosis and the ichthyosis vulgaris gave numbers which were too small for a chi squared test to be applied. Before combining all levels of severity, a test was applied to see whether, despite randomization, one or other treatment had been given to particularly mild or to particularly severe cases. These are shown in Table II.

To confirm that there was no bias in the case of urea, the severity of disease in the patients receiving this treatment was compared with the rest. The chi square was 2·1593; $0{\cdot}30 < p < 0{\cdot}50$; this was not statistically significant. This confirmed that, even in this case, there was no difference in the severity of the disease treated by the different ointments. All groups were therefore comparable.

Handedness

Of the 84 patients in the study 11 were left-handed. These 11 were distributed equally throughout the groups. The incidence of left-handedness was equivalent to that of the population at large (Annett, 1964).

TABLE II.—*Details of Patient Groups in Relation to Disease Severity*

Preparation used	Severity of Disease Mild	Moderate	Severe	Very severe	Total
Oily cream	0	2	6	13	21
Sal. acid	0	5	8	9	22
Urea	0	1	8	13	22
E.45	0	3	9	7	19
Total	0	11	31	42	84

Calculated chi square 6·0382; $0{\cdot}3 < p < 0{\cdot}05$; not significant.

TABLE III.—*Clinicians' Assessment. Total of Both Diseases, All Levels of Severity*

Preparation used	Response to treatment Worsened, or no change	Improved	Marked improvement	Total
Oily cream (observed)	6	14	1	21
Contribution to χ^2	0·7205	0·2500	2·7221	3·6926
Sal. acid	5	14	3	22
Contribution to χ^2	0·0674	0·1060	0·6234	0·7968
Urea	4	8	10	22
Contribution to χ^2	0·0458	1·8201	5·9263	7·7922
E.45	2	13	4	19
Contribution to χ^2	0·8854	0·3315	0·0013	1·2182
Total	17	49	18	84
χ^2	1·7191	2·5076	9·2731	13·4998

Calculated chi squared 13·4998; $0{\cdot}02 < p < 0{\cdot}05$; significant.

Clinicians' Assessment of the Response to Therapy

The clinicians' assessment of the response to therapy is shown in Table III. The statistical analysis of Table III shows that there is a significant difference between the groups, and it is apparent that the discrepancy is due largely to the number of " marked improvements " in patients treated with urea.

Patients' Assessment of the Response to Therapy

Table IV details the patients' assessment of the response to therapy. There was no statistical difference between preparations. Seventeen of 22 patients found the urea cream satisfactory, while 15 of 21 found the oily cream of value. Sixteen of 19 found the E.45 cream and 14 of 22 the salicylic acid ointment to be satisfactory. It is of interest to note the widespread evaluation in Table IV. Provided the questioner, in this case the clinician, recording the response of the patients does not influence them in any way, it is to be expected that there would be a wide range of opinions, since each patient measures the degree of improvement by his own subjective criteria.

DISCUSSION

In a dermatological trial of this type it would seem to be reasonable to take photographs before and after therapy to compare with the clinical assessment. However, Allenby *et al.* (1966) found that photographic assessment was of no more value than the clinical evaluation. For the same reason the views of individual clinicians were not recorded, but the combined opinion of all 3 was used.

It is tempting to suggest that, if a patient withdraws from a trial of treatment, it is because the therapy is ineffective, but we cannot assume this, and such individuals were excluded from the statistical analysis. The patients reported that all 4 preparations were cosmetically acceptable.

The treatment of ichthyosis is still relatively unsatisfactory, and must depend on the enthusiasm of the patient. However, on the basis of this study, a urea cream does seem to control the condition and remove scales better than the other preparations tested. The urea cream is expensive, and since adequate treatment implies life-long use, it must be left to the discretion of individual doctors in relation to the effect in their own patients to judge whether its use is justified.

TABLE IV.—*Patients' Assessment. Total of Both Diseases, All Levels of Severity*

	Response to treatment			
Preparation used	Worsened, or no change	Improved	Marked improvement	Total
Oily cream	6	13	2	21
Contribution to χ^2	0·0455	1·0833	2·4457	3·5745
Sal. acid	8	7	7	22
Contribution to χ^2	0·8693	1·0115	0·1582	2·0390
Urea	5	9	8	22
Contribution to χ^2	0·1007	0·1444	0·6483	0·8934
E.45	3	10	6	19
Contribution to χ^2	0·7848	0·1575	0·1223	1·0646
Total	22	39	23	84
χ^2	1·8003	2·3967	3·3745	7·5715

Calculated chi square, 7·5694; $0{\cdot}20 < p < 0{\cdot}30$. Not significant.

The assessment of the effect by the patients was not statistically significant ($p > 0{\cdot}20$), but this is almost certainly because it was made by inexperienced individuals. It is doubtful if recording this assessment made any significant contribution to the value of the trial.

The numbers were insufficient to allow a statistical separation of the data for the X-linked and autosomal dominant varieties of ichthyosis.

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Case Report

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LINEAR MELORHEOSTOTIC SCLERODERMA

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Summary.—A case of melorheostosis with overlying skin changes is reported. This skin lesion is best termed linear melorheostotic scleroderma to differentiate it from true linear scleroderma. It is characterized by proliferation into the subcutaneous fat of normal-appearing collagen and elastic tissue in lobes and lobules producing an irregular junction between dermis and subcutaneous fat. The association of linear melorheostatic scleroderma with melorheostosis results in a unique symptom complex which is more disabling than melorheostosis alone. It is likely that linear melorheostatic scleroderma represents skin involvement by the same proliferation disorder that produces the bony hyperostosis.

MELORHEOSTOSIS is a rare disorder of bone that is characterized roentgenographically by a linear hyperostosis that appears to flow down the cortex of the bone. The term is derived from the Greek words for member (melos), flow (rhein) and bone (osteon). Since the first description in 1922 by Leri and Joanny, fewer than 200 cases have been described. Of these, 10 cases have had a linear sclerodermatous change overlying the involved bone.

In this communication we report a single case and review the literature concerning the skin manifestation associated with melorheostosis, and suggest that the process differs from true linear scleroderma.

The etiology of melorheostosis is unknown; there is no familial predilection. It occurs equally in both sexes. The extremities are usually affected; however, rare cases involving the trunk and skull have been reported. The most common symptoms are pain in the involved joint and limitation of motion.

Of 131 cases reviewed by Morris *et al.* (1963) 22 (17%) showed cutaneous manifestations. These can be divided roughly into 2 types depending upon the underlying process. (1) The first is associated with tumours or malformations of a vascular or lymphatic nature. The reported vascular lesions consisted of haemangiomata, glomus tumours, A–V aneurysms and vascular naevi; the lymphatic involvement resulted in lymphoedema, trophoedema and ectatic lymphatic vessels. (2) The second variety of cutaneous change has a sclerodermatous or fibrotic component. The terms scleroderma, induration and fibrosis have been used interchangeably in the literature. In the largest single series yet published, (Campbell *et al.*, 1968) it was found that 6 of the 14 cases had changes of this

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sclerodermatous or fibrotic type. Five were reported as fibrosis, 1 as linear scleroderma.

In 1936 Dillehunt and Chuinard published the first case of so-called linear scleroderma and melorheostosis. Since then a total of 10 cases have been reported (Campbell *et al.*, 1968; Dillehunt and Chuinard, 1936; Muller and Henderson, 1963; Clément and Combes-Hamelle, 1942; Sorrel and Quénue, 1948; Thompson *et al.*,

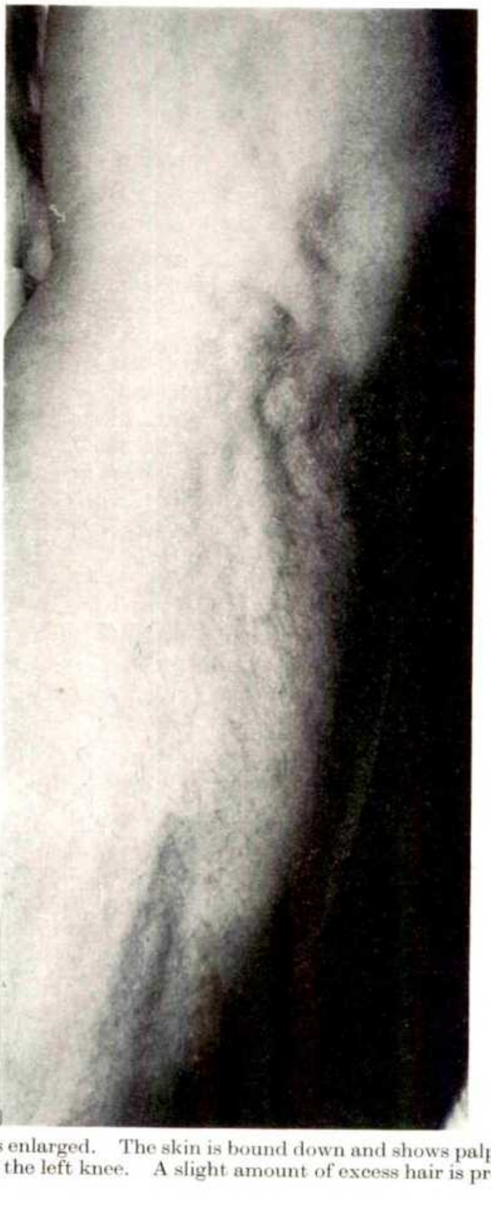

FIG. 1.—The left knee is enlarged. The skin is bound down and shows palpable linear bands on the medial aspect of the left knee. A slight amount of excess hair is present in the area.

1951; Gillespie and Siegling, 1938; Masoteaux and Lamy, 1961; Ernsting, 1966; In these, the histological findings are scanty.

According to Muller and Henderson (1963) there appears to be a distinct symptom complex when linear sclerodermatous skin changes and melorheostosis coincide. This consists of involvement of the knee, pain, frequent shortening of the extremity, and, commonly, dislocation of the patella and muscle atrophy.

Our patient demonstrates this symptom complex and presents a very unusual example of an underlying proliferative process.

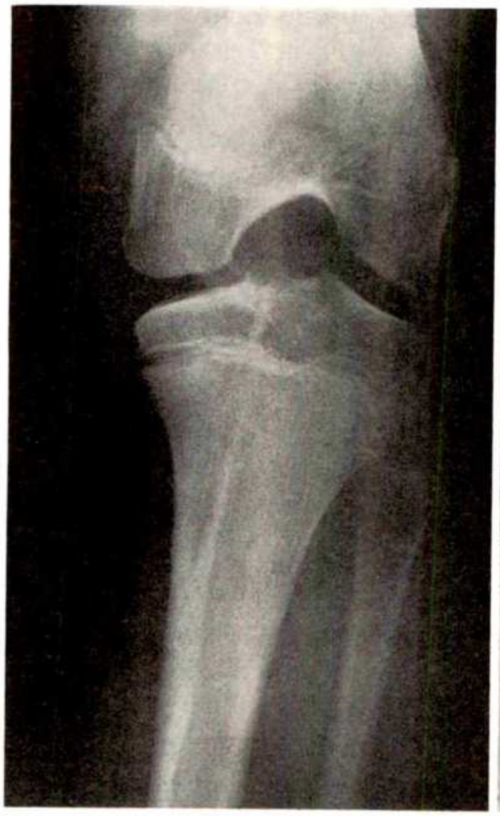

FIG. 2.—A dense streak extends from the tibial epiphysis down to the medial cortex of the proximal tibia. Sclerosis of the patella and soft tissue swelling medial to the knee may be seen.

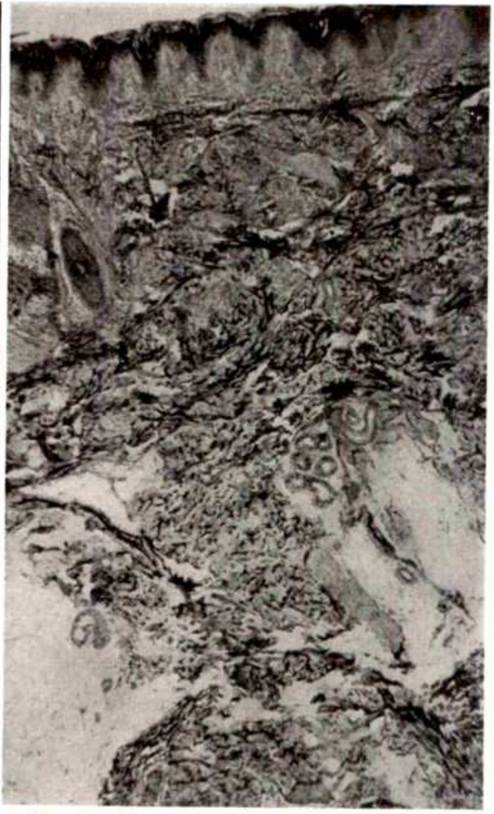

FIG. 3.—There is evidence of proliferation of collagen into the hypoderm with an ill-defined margin. The elastic tissue appears normal throughout (Verhoeff-van Gieson's stain, × 100).

CASE REPORT

The patient was a Caucasoid boy aged 14 whose mother reported thickening and induration of his skin localized to the anteromedial aspect of his left leg and knee at birth. There is no family history of a similar condition. At the age of 10 pain developed in the knee. Melorheostosis was diagnosed at that time by radiological examination. In the subsequent 4 years the pain grew progressively worse and he was admitted to St Luke's Hospital Centre in February 1971 for consideration of a lumbar sympathectomy;

however, a sympathetic block did not relieve his pain, and it was felt that the operation was not indicated.

Physical examination (Fig. 1).—Abnormal findings were limited to the left lower extremity. The knee was enlarged, with limitation of motion in the joint. The skin overlying the knee was thickened; the thickening extends downwards in a linear fashion over the left anterior tibial surface. The involved skin was normally pigmented, but felt slightly roughened. It was bound down and nodules and linear bands could be palpated. A small amount of excess hair was present in the area.

Laboratory data.—Blood count, urinalysis, serum calcium, serum glutamic oxalacetic transaminase and lactic dehydrogenase were normal. The serum phosphorus and alkaline phosphatase were slightly elevated, but not abnormally for an adolescent male. The immunoelectrophoresis was normal. Anti-nuclear antibody and rheumatoid factor tests were negative.

Radiological examination (Fig. 2).—X-rays of the left knee revealed a dense streak extending from the tibial epiphysis down to the medial cortex of the proximal tibia. There was sclerosis and downward displacement of the patella. Soft tissue swelling could be seen medial to the medial epicondyle of the femur. Measurements of the bones of the extremities on x-ray film showed a shortening of 1·5 cm of the left femur and 1·4 cm of the left tibia when compared to the right. X-ray examination of the chest was normal.

Histology (Fig. 3).—A biopsy of the skin of the involved area was compared with a biopsy from a comparable site of the uninvolved opposite leg. In both, the epidermis was normal and the rete ridges were intact. The cutaneous appendages were normal in appearance and were not reduced in number. The collagen appeared normal with H. and E. and did not stain metachromatically with alcian green/periodic-acid Schiff. An elastic tissue stain with Verhoeff-van Gieson's stain showed no decrease in the elastic tissue. In the involved area there was proliferation of normal-appearing connective tissue in strands and lobules into the subcutaneous fat. This was not found in the section of normal skin, where a sharp demarcation of the dermis from the subcutaneous fat was present.

DISCUSSION

The changes in the skin of this patient are probably related to the underlying melorheostosis. The changes in both the skin and the bone are linear and one overlies the other. There would appear to be some special relationship between the melorheostosis and the overlying "sclerodermatous" process, as suggested by Muller and Henderson (1963).

The histological changes in this patient are not consistent with linear scleroderma, although other cases with the same clinical signs and symptoms have been called scleroderma. Fleishmayer *et al.* (1971) have demonstrated that sclerodermatous skin will show histologically replacement of subcutaneous fat with abnormal-appearing collagen in which there is a decrease of elastic tissue. None of these histological features was found in our patient. Instead there was a downward proliferation of normal-appearing collagen into the subcutaneous fat. A biopsy of skin from the opposite knee showed a smooth demarcation at the dermal–subcutaneous fat junctions as one would expect of skin over a movable joint. It is probable that the proliferation of collagen in lobules and nodules into the subcutaneous fat accounts for the clinical findings of bound down skin and limitation of motion of the affected joint. A gritty feeling on punch biopsy is produced by differences in texture between alternating collagen and fatty tissue. This gritty feeling was present in our patient and has been reported before (Thompson *et al.*, 1951).

We feel that, although there may be gross similarities, the process is not true linear scleroderma. Rather, it is essentially a localized proliferative disorder. This proliferation is demonstrated clinically by enlargement of the knee, palpable thickening of the skin, excess hair in the area, hyperostosis of bone, and histologically by downward proliferation of collagen. Further evidence that this is a proliferative disorder is its tendency to progress during periods of active growth (adolescence), but to remain static during periods of no growth. In addition, the hyperplastic lymphatic or blood vessel tumours that are sometimes associated are further evidence of the proliferative nature of the process.

The separation from true linear scleroderma is established by a histological picture showing proliferation of normal-appearing collagen in lobes and lobules in the subcutaneous fat. In scleroderma abnormal-appearing collagen replacing normal collagen is seen, and a linear, uninterrupted junction between the dermis and subcutaneous is maintained. The ANA antibody test, which may be positive in focal childhood scleroderma, (Hanson *et al.*, 1970) was negative in our case.

Our findings suggest that a more accurate term should be given to this syndrome than " melorheostosis with scleroderma ". Since the term is firmly established in the literature, we do not recommend that it be dropped, but we suggest a modification. A less confusing designation of the skin changes is " linear melorheostotic scleroderma ", implying a process distinct from true linear scleroderma.

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Comment

THE PATHOGENESIS OF LICHEN PLANUS

Lichen planus recently celebrated its dermatological centenary (Samman, 1969). Although lichen planus has such distinctive clinical and histopathological features, the aetiology of the condition is still virtually unknown. The classical histopathological features of lichen planus were clearly described over 60 years ago (Darier, 1909). Sabouraud (1910) considered that lichen planus was primarily a dermal disease and that the epidermal changes were secondary. Kyrle (1925) felt that the colloid bodies in the dermal papillae were derived from degenerating collagen bundles. In 1957 Thyresson and Moberger suggested that the pathological process primarily affected basal epidermal cells and that the inflammatory infiltrate developed as a secondary manifestation. These workers clearly showed that the colloid or " Civatte " bodies were derived from degenerating epidermal cells and attributed this change to a virus infection.

Lichen planus has long been considered to have a possible infectious aetiology and certain clinical evidence has tended to support this view (e.g. Barber, 1947; Depaoli, 1970). Lennhoff (1948) claimed to have demonstrated delicate spirochaetes in frozen histological sections of lichen planus but since then this observation has never been confirmed. In an ultrastructural study of 4 lichen planus papules, Brody (1965) described the presence of rod-like structures between lower epidermal cells and also around capillaries in the dermal papillae. Brody considered that these rod-like structures were bacteria, and in a later study (Brody, 1969) found the same type of structure, but in only 7 out of 42 lichen planus papules. Swanbeck and Thyresson (1964) reported the presence of intranuclear laminated particles in epidermal cells and suggested that they could be viruses. Johnson and Fry (1967) described two types of intranuclear structures, one of which was lamellated in form. These lamellated particles measured from 260 to 1200 mμ which the authors pointed out were usually considerably larger than the largest known viruses (range 350–400 mμ). Similar intranuclear inclusions have also been noted in kerato-acanthoma (Zelickson and Lynch, 1961; Zelickson, 1962 and Gay Prieto *et al.*, 1964) and psoriasis (Brody, 1962a, b), suggesting that they are a non-specific manifestation due to a general disturbance of cellular function. Bouteille *et al.* (1967) have also suggested that intranuclear inclusions are related to cellular hyperactivity, whether this was physiological, drug induced, or due to a specific disease process. Sarkany and Gaylarde (1970) also found a large lamellated intranuclear inclusion body (300 to 1200 mμ) in lichen planus papules, but found that such bodies were not limited to epidermal cells. They were occasionally able to demonstrate the presence of similar intranuclear inclusions in normal human skin, whilst they were also seen in larger numbers in basal-cell carcinoma and kerato-acanthoma. Sarkany and Gaylarde demonstrated the presence of a smaller type of intra-nuclear inclusion particle (100 to 300 mμ), similar in size to that of a virus. They concluded, however, that " their rarity, arbitrary distribution in epithelial cells of the various layers, and absence in many cases altogether, are against the likelihood of their being viruses ". Goltz and Hult (1963) showed that the colloid bodies stained negatively with Feulgen's reaction, and by other staining procedures they were unable to detect DNA or RNA. Goltz and Hult therefore discounted the possibility that the colloid bodies were of viral origin, and concluded that they represented degenerate epidermal cells. Furthermore, Fry and Withers (1968) failed to find any evidence of virus or mycoplasma infections in lichen planus by means of various cultural and serological techniques.

Other aetiological possibilities (e.g. auto-immune mechanisms and psycho-somatic factors) have also been postulated for lichen planus, but little supporting evidence has been forthcoming so far. Midana and Ormea (1958) claim to have demonstrated a significant increase of nervous tissue in the papillary dermis of early lichen planus lesions. This observation has not, so far, been confirmed by others, so its significance must remain in doubt. Graciansky *et al.* (1958) have claimed that some lichenoid reactions (e.g. from drugs and colour film developers) are identical clinically and histologically to lichen planus. It is therefore important to remember these reactions when the aetiology of the condition is being considered.

Until recently surprisingly little attention has been paid to the metabolic function of the affected epidermal cells or the kinetics of the epidermis in lichen planus papules. Black and Wilson Jones (1972) performed detailed enzyme histochemical studies on 15 biopsy specimens of acute lichen planus. The most striking finding was an apparent marked depression of respiratory enzyme activity in the affected epidermis, using techniques for the demonstration of succinic dehydrogenase, cytochrome oxidase and NAD diaphorase activity. In these areas the individual epidermal cells appeared to be increased in size and contain larger amounts of cytoplasm. Several authorities (e.g. Lever, 1967; Pinkus and Mehregan, 1969) have suggested that epidermal-cell turnover is decreased in lichen planus. Hendren and Pinkus (1954) showed that when mitoses in the epidermis were suppressed by applications of thorium X, the epidermal cells became larger and the proportion of maturing cells increased. In a current study of the epidermal kinetics in lichen planus (Marks, Black and Wilson Jones, in preparation) using tritiated thymidine, it has been shown that where the lower epidermis consisted of large pale keratinocytes no labelling was seen. On the other hand, at the edges of the lichen planus papule, labelling was observed in spindle-shaped keratinocytes and sometimes the labelling index at these sites was increased. It is likely that this phenomenon represents the healing process, in that keratinocytes migrate from the periphery and repopulate the lesion. Specimens incubated with tritiated histidine showed a broadened epidermal area of incorporation compared to uninvolved sites. These results indicate that the epidermis of the lichen planus papule has lost its generative capacity due to erosion of the basal cell layer and that it tends to behave metabolically like the upper part of the epidermis and the granular cell layer.

Black and Wilson Jones (1972) also showed that the numbers of dopa-positive melanocytes were markedly decreased in active lichen planus papules, indicating that the melanocytes are also involved and probably destroyed by the same process which affects lower epidermal cells. No increase in various hydrolytic enzymes associated with lysosomal activity could be demonstrated in areas where basal cell necrosis was a marked feature. Johnson and Fry (1967) have demonstrated a decrease in the numbers of tonofibrils and desmosomes in affected lower epidermal cells. In a recent ultrastructural study Sarkany and Gaylarde (1971) showed that liquefaction degeneration of basal cells could occur in the absence of an inflammatory infiltrate and without any disruption of the basement membrane. Rupture of the basement membrane did occur in the presence of a marked dermal infiltrate, and subsequently attempts at repair led to the formation of several new basement membranes. These findings support the concept that in lichen planus the primary pathology affects basal epidermal cells. Although Ueki (1969) has demonstrated that the colloid bodies in lichen planus contain immunoglobulins, βIC-globulin, albumin and fibrinogen, it is likely that their deposition is a secondary phenomenon. Lymphocytic infiltrates show little or no hydrolytic enzyme activity (Braunstein *et al.*, 1962), but in lichen planus the dermal infiltrate shows a much more intense activity in some areas (Berger *et al.*, 1969; Black and Wilson Jones, 1972) and it is likely that this represents macrophages.

Glickman *et al.* (1964) have suggested that the characteristic violaceous hue of the lichen planus papule is due to an unusual radial arrangement of capillaries at the edge of the lesion. The microscopic changes responsible for the clinical appearance of Wickham's striae are probably related to a focal increase in lichen planus activity (Summerly and Wilson Jones, 1964). Ryan (1966; 1971) feels that the classical pallor of Wickham's striae may be due to a focal loss of capillaries and that impaired nutrition may contribute to the epidermal changes. However, Black and Wilson Jones (1972) have shown that the capillaries appeared to be distributed normally in areas of focal activity with both alkaline phosphatase and ATPase reactions, so it seems likely that in these areas the capillaries remain viable.

Despite these recent studies we remain largely ignorant of the aetiology of lichen planus or why the lower epidermal cells bear the brunt of the disease process. Biochemical studies at a cellular level may give further clues to the earliest changes in affected epidermal cells.

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TOULOUSE 1970

THE 1970 Franco-British meeting was held from May 27th to 31st in the ancient city of Toulouse, capital of Languedoc. The British party had been kindly invited to attend the National Congress of the French Dermatological Society on May 28th. The meeting took place in the Palais des Congrès where the dermatologists of both countries were welcomed by the Prefect of the Region, the Dean of the Medical School and the President of the French Society, M. le Coulant.

For those who were not bilingual simultaneous translation was provided of such excellence that useful discussions from the audience were possible. One feature of the meeting was that the time allotted for discussion exceeded that of the communications, an innovation we might copy.

The second day was given over to the Franco-British scientific programme and the communications from both countries aroused interested discussions which were conducted briskly under the guidance of the French and British Presidents.

On May 30th, an impressive collection of interesting patients was shown in the Solac Laboratories and it was apparent that Professor Bazex and his colleagues had gone to great lengths to prepare this programme.

The arrangements made for social functions by Professor and Madame Bazex and their colleagues in Toulouse were superb. A visit was paid to the Toulouse-Lautrec Museum at Albi and we were entertained on one evening in the historical surroundings of the Hôtel Dieu St Jacques and on another evening at the Hôtel Comte de Toulouse. The official dinner was held in the Priory of Malta where a memorable spectacle was the performance of folk dancing in the floodlit courtyard. Singing troubadours entertained the guests during dinner.

The meeting ended with a tour of Gascony and a luncheon at which several of the British party were honoured by admission to the order of the Musketeers of Armagnac.

The following papers were read. They have been published in French in *Bull. Soc. fr. Derm. Syph.* (1970) **77**, 669–716.

I. B. Sneddon (Sheffield), " Harmful Effects of Topical Steroids ". The changes induced by the topical application of fluorinated corticosteroids were described, and illustrated by numerous clinical examples. The undesirable changes most frequently encountered were atrophy and telangiectasia, especially on the face, and the " perioral dermatitis " syndrome. The course of ringworm infections may be so modified that diagnosis is difficult and the healing of leg ulcers may be delayed.

B. Duperrat and L. Ficheux (Paris), " Cutaneous and Vulval Endometriosis ". The authors reported their personal experience of 25 cases of cutaneous or vulval endometriosis; in 10 the lesion was in operation scars, in 6 at the umbilicus, in 6 on the vulva and in 3 in the inguinal region. Regular menstrual enlargement and haemorrhage is a characteristic but inconstant clinical feature.

J. Marks and S. Shuster (Newcastle), " Some Dermatological Aspects of Diseases of the Small Intestine ". The interrelations between diseases of the skin and of the small intestine were discussed in general terms and the diseases were then classified into 4 groups. In Group I the intestinal disease causes the skin changes as in coeliac disease or tropical sprue. In Group II the skin disease causes the steatorrhoea, as in dermatopathic enteropathy. In Group III the same disease affects both skin and small intestine as in polyarteritis nodosa, and in Group IV there is an indirect relationship between the skin eruption and the disease of the small intestine, as in dermatitis herpetiformis.

A. Basset, J. Maleville, H. Bergoend, E. Grosshans, E. Heid and J. Zimmer (Strasbourg),

" Drug Eruptions ". The authors reported on the work of their team over a period of 5 years on 3290 patients with drug eruptions. Apart from the study of the clinical and histological features, the investigation included Shelley's test, skin tests (found to be of limited value) and, in a number of cases, lymphoblastic transformation tests.

J. H. Pettit (Kuala Lumpur), " *Mycobacterium ulcerans* Infections ". The climatic conditions prevailing in the countries in which *M. ulcerans* infections had been reported suggested to the author the probability that such infections also occur in Malaysia. He reported cases personally encountered there and discussed their diagnosis and treatment.

C. Huriez and J.-C. Allart (Lille), " Arterial Ulcers ". The role of the arterial factor in leg ulcers was probably underestimated in the past. In 1945, 2% of leg ulcers in Lille were regarded as arterial in origin; the percentage had risen to 15% by 1969. The clinical and radiological features of 252 arterial ulcers were described. The incidence of arterial ulcers has been increased by cigarette smoking and their prophylaxis should therefore begin in adolescence.

S. Alexander (London), " Lichen Amyloidosus ". Twenty-five cases of lichen amyloidosus were seen over a period of 3 years. Investigation of these cases suggested the possibility that lichen amyloidosus may herald the subsequent development of multiple myelomatosis.

M. Black (London), " Lichen Amyloidosus ". A summary was given of the clinical, histological and histochemical findings in 13 patients with the macular, pigmented form of lichen amyloidosus.

W. M. Stewart and P. Lauret (Rouen), " Progressive Vascular Fistulae and the Pseudo-Kaposi Syndrome ". The study of 2 patients formed the basis of an investigation of arteriovenous fistulae presenting atypically with essentially cutaneous lesions, which may simulate Kaposi's sarcoma.

Books

A Treatise of Skin. Vol. 1—Biophysical Properties of the Skin. Edit. H. R. ELDEN (1971). New York: Wiley-Interscience. pp. 654, 183 figures, tables. Price £16.50.

This volume is the first of a new review series on the physics, chemistry and biology of skin. It is clear from a study of the contents of the chapters of the present volume that extensive theoretical and practical work has been done on the components of skin since Rothman published his classic volume on physiology and biochemistry of the skin in 1954. This is particularly true of collagen, the main physical structure of the dermis. A considerable part of this book is concerned with the melting and deformation properties of this material. Aggregation phenomena of collagen are dealt with by J. M. Cassel, cross-links in elastin and collagen are discussed by Frank S. Labella and sorption of water by collagen is considered in detail by the late J. R. Kanagy. The last chapter is a review by D. R. Cooper on the effects of irradiation of soluble collagen, including effects of ultraviolet light, a topic of some importance since it is now established that a significant proportion of ultraviolet radiation penetrates through intact epidermis into the deeper tissues. There is a good chapter on the structure of elastin fibres by D. A. Hall of Leeds and a delightful account of the mechanical properties of skin in relation to its biological function and chemical components by R. D. Harkness.

However his statement on p. 397 that " the hypothetical function of the fingerprint, epithelial ridges and sweat glands in gripping seems not to have been investigated " is not quite correct, and anyway once it is known that skin wetted by moderate sweating in these areas has a greatly increased co-efficient of friction the obvious value of this physiological change in situations of fight or flight seems hardly worth discussing. In the same context the advantages of a skin rendered slippery by an emulsion of lubricant fluids from the sebaceous and sweat glands (apart from the soles and palms) is not discussed. There is no discussion in this chapter or elsewhere of modelling of collagen by collagenases although there is extensive information offered on the effect of stress on collagen and whole skin specimens. Nor does any author considers the effect of cyclically repeated stresses with consequent strain hardening and the induction of fatigue phenomena—surely topics which are of profound importance in the living organism.

The chapter by Kirk C. Hoermann on the optical properties of skin and its biochemical substituents is of great interest; in it he discusses self-luminescence of skin, both fluorescence and phosphorescence. Self-fluorescence in particular is of great current interest in the field of fluorescent antibody techniques, and there is a useful discussion of this. The chapter by Robert Edleberg on the electrical properties of skin is good material for somebody about to enter work in this field. In his discussion on the measurement of spontaneous potentials however it might have been valuable for there to have been some information given on the design and choice of stable electrodes since this would appear to be a critical factor in the accurate measurement of these small potentials.

This book is a compendium of monographs by experts presenting their material for the most part in a very detailed and technical manner; I cannot imagine there are many clinical dermatologists who would find this book of much value to them unless they happen to research in one of the specialized fields covered in the chapters. For the biologist interested in the skin of man or other species, and who also has a yen for physics or physical chemistry, the volume should prove of great interest, but he will need a fair knowledge of mathematics and mathematical physics to keep up with some of the authors. It is the sort of book that I cannot help feeling we will have to come to grips with in dermatology sooner or later. Probably heads of departments should buy it for their libraries so that the brighter registrars can borrow it and base on it some seminars for their elders' edification.

We are " cordially invited by the editor to share in planning the next volumes of this continuous series on the basic properties of skin and its components ". I have two suggestions:

1. that the epidermis should be fully considered in at least one of the volumes (including of course the topic of friction),
2. that the effect of mechanical fatigue on skin and its components is likely to be of direct relevance to much of our clinical and laboratory work on the physical properties of skin.

STANLEY COMAISH

Cutane Paraneoplastische Syndrome. J. J. HERZBERG (Ed.) (1971). Stuttgart: Fischer. Pp. 104, 17 illus. Price £4.32.

In the summer of 1969 a combined meeting was held in Bremen of the Dermatological Societies of North West Germany and of Hamburg. Two days were devoted to a symposium on paraneoplastic cutaneous syndromes. The proceedings of the symposium have been edited by Professor Herzberg of Bremen. The 16 papers present comprehensive and well documented (with one exception) and critical reviews of the now extensive literature on the skin markers of malignancy. Most of the papers are in German with short English summaries, but 3 are in French and 2 in English.

The contributors have covered the ground so thoroughly that even the most learned of readers is unlikely to acquire no new facts from this well edited volume. Particularly welcome are the accounts of paraneoplastic acrokeratosis by Bazex and his colleagues, still too little recognized outside France, of acquired ichthyosis by Kuske, pruritus and erythroderma by Thies and of skin infections by Meyer-Rohn. This small book is highly recommended.

ARTHUR ROOK.

Acne Vulgaris. SAMUEL B. FRANK (1971). Springfield: Thomas. Pp. 430, 50 illus. Price £10.13.

Dr Frank regularly gives a course of lectures on acne vulgaris in the Department of Dermatology of the New York University School of Medicine. His present monograph is intended to provide a comprehensive review of acne vulgaris with " a reasonably complete bibliography ".

A short introductory section is followed by sections on the pathogenesis of acne (124 pages), histopathology of acne (10 pages), management of acne (192 pages) and differential diagnosis (48 pages). The disproportionate length of the section on management reflects the difficulty of managing acne effectively in the absence of a specific treatment and the continuing need for time consuming long term supervision and multiple therapeutic approaches.

The literature is in general competently reviewed but the author too often fails to give sufficient detail to enable the reader to decide to what extent investigations leading to conflicting conclusions are in fact comparable. The discussion of hereditary factors is one of many examples of this deficiency. Similarly, significant detail is omitted in parts of the account of hormonal factors, as when " androgens " are referred to rather than the individual hormones. The citation of references by numerals has advantages but it distracts the reader when he is forced to turn frequently to the references to ascertain the years of publication of apparently contradictory findings.

Although the author does not claim that his bibliography is complete, few important references have been overlooked and the coverage of the literature is refreshingly international. It could indeed be claimed that the net has been cast too widely and that had some of the worthless or repetitive papers been omitted, space could have been found for a far more critical and detailed analysis of the important ones. However this book is nevertheless a valuable contribution and all who are interested in acne should be grateful to Dr Frank for compiling it.

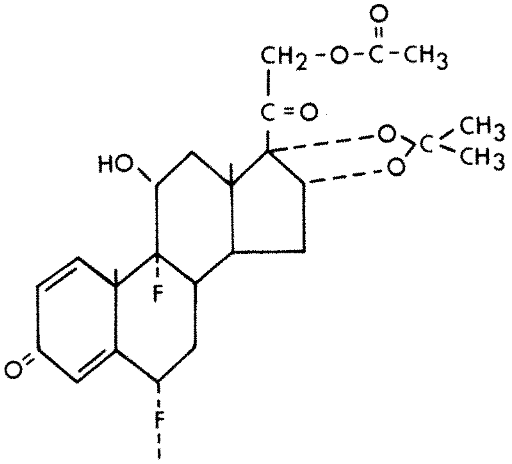
ARTHUR ROOK

Br. J. Derm. (1972) **86**, 310.

Correspondence

" Metosyn " in Psoriasis and Eczema

SIR,—We have recently carried out a clinical trial with a new topical corticosteroid, fluocinonide, FAPG (" Metosyn "). This steroid is 6 alpha, 9 alpha difluoro 11 beta, 16 alpha, 17 alpha, 21-tetrahydroxy-pregna-1, 4-diene-3,20 dione 21-acetate 16,17-acetonide with a structural formula:



The base (FAPG) consists of Stearyl alcohol; Polyethylene glycol 6000; propylene glycol; 1,2,6-hexanetriol.

The fluocinonide is in solution in its optimum concentration in this preparation which has the properties of both a cream and an ointment.

The blanching test had previously shown fluocinonide to be superior to betamethasone 17-valerate (Stoughton, 1969; Coldman *et al.*, 1971; Sarkany *et al.*, unpublished) and this led us to investigate this preparation clinically. In a paired double-blind trial 140 patients were treated using " Metosyn " with and without polythene occlusion. Of these 44 were cases of psoriasis and 96 had various types of eczema. The material was applied to the appropriate areas and side according to the coding, 2, 3 or 4 times daily, with or without occlusion at night and the results assessed at the end of the first week and again at the end of the second.

The results of treatment without occlusion in patients with psoriasis and eczema are shown in Table I.

TABLE I.—*Comparison of Effect of Fluocinonide FAPG and Betamethasone Valerate in Patients Treated without Occlusion*

Clinical conditions	Number of cases where fluocinonide better	Number of cases where betamethasone better	Number of cases showing no difference
Psoriasis	16	2	7
Eczema	46	25	14
Total	62	27	21

Forty-six patients with eczema responded better to " Metosyn " against 25 who responded better to betamethasone valerate. The difference is statistically significant at the 2% level. Of 25 patients with psoriasis 16 responded better to " Metosyn " against 2 who responded better to betamethasone valerate. The difference is statistically significant at the 1% level.

TABLE II.—*Comparison of Effect of Fluocinonide FAPG and Betamethasone Valerate in Patients Treated with Occlusion*

Clinical conditions	Number of cases where fluocinonide better	Number of cases where betamethasone better	Number of cases showing no difference
Psoriasis	7	2	10
Eczema	4	3	4
Total	11	5	14

Of the 30 patients with psoriasis and eczema treated with occlusion 11 responded better to " Metosyn " against 5 who responded better to betamethasone valerate. This difference did not reach statistical levels of significance although the trend was in favour of " Metosyn ".

Table II shows results of treatment with polythene occlusion.

We have not encountered any serious side effects in the treatment of this series and we have so far been impressed by the absence of secondary infection and allergic reactions to the base. It would appear that " Metosyn " marks yet another advance in topical steroid therapy and that it is the topical steroid of choice in the management of psoriasis.

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SARKANY, I., HADGRAFT, J. W. & BAKER, B. Unpublished data.

The Effect of a Medicated Wash on Acne, Sebum Excretion Rate and Skin Surface Lipid Composition

SIR,—There is need for more effective topical therapy in acne vulgaris. An approach to this problem should take into account factors which might lower sebum excretion rate, modify skin surface lipid composition, skin flora or the resistance to sebum outflow all of which may be important in the pathogenesis of acne (Cunliffe and Shuster, 1969a). We have investigated the clinical effectiveness of a medicated wash containing hexachloraphane and propylene phenoxytol in acne and on the sebum excretion rate and skin surface lipid composition. This study was prompted by both *in vitro* investigations on this wash (Rodger and Breach, 1972) and clinical observations of its efficacy in acne (Keszces, 1971).

The effect of the medicated wash* used topically twice daily was studied in 10 patients, 6 females and 4 males aged between 12 and 17 years with mild or moderate acne. At the initial and subsequent visits, which were at one monthly intervals, the patients were assessed clinically and their forehead sebum excretion rate determined by the modified method of Strauss and Pochi (1961) (Cunliffe and Shuster, 1969b). The composition of forehead surface lipid was determined semi-quantitatively for squalene, wax esters, triglycerides, free fatty acids and cholesterol (Cotterill *et al.*, 1971).

Clinical improvement was seen in 4 patients after 1 month, in 3 patients after 2 months and in 8 patients after 3 months of therapy ($P = 0{\cdot}025$). One patient had defaulted at this stage from the trial.

The wash had no effect on the sebum excretion rate. There was a significant decrease in the percentage of surface lipid wax esters after 2 months of therapy but this effect was not seen after 3 months. There were no significant changes in the other surface lipid constituents studied.

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* Supplied by Richardson-Merrell Ltd., 12 Brewery Road, London N.7.

We conclude that this preparation is effective in patients with mild or moderate acne after 3 months of therapy. Because we found neither changes in the sebum excretion rate nor consistant changes in surface lipid composition then this medicated wash may modify other factors important in the pathogenesis of acne vulgaris.

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St James' (University) Hospital, Leeds — J. A. Cotterill
Harrogate General Hospital — B. Williamson

We thank the Board of Governors of United Leeds Hospitals for financial support, and Dr P. Thackray for his advice.

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Dapsone

Sir,—Your correspondent Dr Peter Ashurst (*Br. J. Derm.*, **85**, 499) is right to draw attention to the fact that I.C.I. have ceased making Dapsone tablets. I too have had innumerable enquiries from sufferers from dermatitis herpetiformis and from their doctors and chemists. However, the information which Dr Ashurst has passed on is unfortunately out of date. Thomas Marns have been taken over by Arthur H. Cox, 93 Lewes Road, Brighton BN2 3QJ, 'phone Brighton 63084. I am informed by the latter that they market tablets of 100 mg, 50 mg and 10 mg, strengths which will be welcome to many dermatologists who prescribe doses below 50 mg daily.

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Br. J. Derm. (1972) **86**, 313.

Quarterly Review

DRUG REACTIONS XVII

Cutaneous Reactions to Barbiturates, Chloral Hydrate and its Derivatives

BARBITURATES

More than 20,000,000 prescriptions for barbiturates are dispensed each year in England and Wales. It is not possible to give estimates for individual products as many proprietary preparations are mixtures containing barbiturates.

Barbiturates probably account for more drug rashes than any other drug. Although the actual incidence is low, rashes due to barbiturates are seen frequently, 1–3% of patients taking phenobarbitone developing skin reactions. Barbitone, phenobarbitone and other slowly excreted, long-acting barbiturates are generally believed to cause more rashes than the more quickly excreted short-acting barbiturates, e.g. thiopentone sodium (Tattersall, 1965; Grant Peterkin, 1956), but no unequivocal evidence for this exists (Rook *et al.*, 1968). The commonest reactions found with this group of drugs are *allergic* in nature, the most frequent and typical being a maculopapular eruption which may be preceded by pruritis. It is widespread accompanied by fever and lymphadenitis. Less commonly a scarlatiniform rash occurs (Davison, 1943). On cessation of therapy the rash usually fades quickly, but if the drug is continued the consequences may be serious and fatal. Sneddon and Leishman (1952) described 4 severe reactions of this type in which 2 patients died. Exfoliative dermatitis has also been reported, as a primary eruption or following morbilliform or scarlatiniform eruptions (Potter and Whitacre, 1944).

Urticaria and serum sickness occur, but are reported less frequently with the longer acting barbiturates than with thiopentone sodium in which an incidence of 3 cases after 15,000 injections was reported by Evans and Gould (1952). One of their cases showed urticaria, erythema and angioedema the distribution of the reaction being in light exposed areas. Photosensitivity is not common after barbiturate therapy but is recognized. Stryker (1939) described a child aged 2 who developed an exfoliative dermatitis after 6 weeks' barbiturate administration. Four months later the child developed an acute dermatitis of the exposed areas after sun exposure and was found to excrete barbitone and porphyrins in the urine.

Barbiturates are known to exacerbate acute intermittent porphyria and porphyria variegata. Occasionally, it is very difficult to exclude porphyria variegata in patients who have skin lesions of porphyria cutanea tarda, and if there is any doubt barbiturates should be avoided.

Cross sensitivity between the various barbiturates has not been fully investigated but if one barbiturate, e.g. thiopentone, has caused morbilliform rash and angioedema another barbiturate, e.g. hexobarbitone, does not necessarily cause a similar reaction (Evans and Gould, 1952). However, if barbiturate sensitivity is suspected, related drugs should be prescribed with caution.

Anaphylaxis is more commonly reported with the short-acting preparations. Patients who receive thiopentone in multiple general anaesthetics, e.g. repeated cystoscopy, electroconvulsive therapy, orthopaedic or plastic procedures, appear to be at special risk (Clark and Cockburn, 1971).

Purpura has been reported by Grant Peterkin (1946). His patient developed a punctate purpuric rash after thiopentone, which faded after one week. This rash recurred on 2 subsequent occasions following further thiopentone anaesthetics.

Erythema multiforme occurs occasionally (Hunter, 1943; Rook *et al.*, 1968) although Grant Peterkin (1956) found barbiturates were the commonest cause of the Stevens–Johnson syndrome in which mucosal involvement was often severe. Further support for this was given by Sneddon (1966) who found that phenobarbitone was the commonest cause of the Stevens–Johnson syndrome in a group of patients with ulcerative colitis.

A reaction resembling systemic lupus erythematosus was reported in a woman aged 67 who had an erythematous rash with a raised papulo-vesicular margin on her cheeks, forehead and neck which became more extensive in a hot summer. There was no follicular plugging. No histological evidence of lupus erythematosus was found. The patient had taken 60 mg of phenobarbitone per day for one year. The rash cleared 2 months after the drug was stopped (Grant Peterkin, 1951). No further cases have been reported.

Fixed drug eruptions due to barbiturates are well documented (Meredith, 1934). Savin (1970) showed that barbiturates are the commonest cause of this type of rash.

Toxic epidermal necrolysis is rare but has been recorded in an epileptic boy aged 4½ who had a previous history of allergy to phenytoin (Vandvik, 1967).

Jaundice is uncommon but Pagliaro *et al.* (1969) reported cholestatic jaundice following barbiturate administration with exacerbation of jaundice recurrence of fever and a skin eruption following a rechallenge with phenobarbitone.

Phenobarbitone may induce the formation of drug metabolizing enzymes which interfere with the activity of other drugs including griseofulvin, phenytoin, strychnine and coumarin anticoagulants.

Barbiturates may also produce rashes which are *toxic* rather than allergic in nature. These are found in 4% of patients with barbiturate induced coma and in 40% of those that die (Mandy and Ackerman, 1970). The rashes consist of dusky erythematous plaques, tense vesicles, bullae and deep, slowly healing ulcers, on occasions resembling burn eschars (Harris, 1971). These are found mainly at sites of trauma, pressure or where 2 limbs or digits are in contact. Blisters may appear minutes after trauma and persist for days. The pathogenesis of these blisters is uncertain but because of the sites affected, pressure and hypoxia associated with circulatory changes are regarded as of importance.

Histological examination shows necrosis of the epidermis and also necrosis of eccrine sweat gland epithelium, subepidermal bulla formation and intraepidermal spongiosis; located mainly adjacent to the intraepidermal sweat ducts, with little dermal damage as indicated by only a few polymorphonuclear leucocytes (Leavell, 1969; Mandy and Ackerman, 1970). Experimental studies have shown similar findings. Raab (1962) observed the effect of injecting 50–70 mg of phenylethylbarbiturate intramuscularly into guinea-pigs, their limbs were subjected to mild compression during 3 days of deep anaesthesia and biopsies were taken from these areas at 24, 48, and 84 hours. The resulting picture closely resembled barbiturate ulcers in man. Two stages were noted: 1. Blister formation and exudation. 2. Necrosis. Results suggested that subepidermal blisters were caused by direct toxic damage to the basal cells. Subsequently the connective tissue formed papillae leading to sloughing of the epidermis.

These changes led Beveridge (1970) to speculate that the eccrine sweat glands might be playing a part in the formation of these bullae by excreting barbiturate, with local blockage, e.g. where 2 limbs or fingers were in contact, leading to further increased concentration. Gröschell *et al.* (1970) have reported a case of barbiturate overdosage where they found barbiturates in blister fluid. Hyperthermia may also play a part in the formation of bullae. As one third of the body temperature is eliminated by the sweat glands, pathological changes in the sweat glands could contribute to the hyperthermia seen in barbiturate coma (Leavell, 1969). The bullae were originally believed to be pathognomic of barbiturate induced coma. However, similar clinical and histological lesions are reported in carbon monoxide poisoning (Leavell *et al.*, 1969) and drug overdosage due to methadone, imipramine, glutethimide, meprobamate and nitrazepam (Ridley, 1971).

Blau *et al.* (1962) have reported keloid formation at sites of bullae formation in 3 white women. Patients who have suffered from barbiturate induced coma and developed bullae due to toxic effects need not avoid barbiturates later for fear of developing allergic reactions, they are no more likely to develop reactions than the general population.

CHLORAL HYDRATE

Chloral hydrate is the oldest of the hypnotic drugs and has been used extensively. Reactions are rarely reported but tend to be overlooked as this drug is not suspected. The commonest reported reactions (Christianson and Perry, 1956) include erythema, predominantly affecting the face, neck and chest, scarlatiniform eruptions (Morrow, 1893), urticaria, angioedema, purpura, eczema, bullous lesions and erythema multiforme. Watson (1878) had previously described a reaction to chloral hydrate which could be classed as a fixed drug eruption.

Ethchlorvynol may be regarded as a condensation product of chloral hydrate. Auerbach (1965) reported a fixed drug eruption affecting the abdomen and thighs of a woman aged 41. The eruption recurred in the same sites when the patient self-administered the drug again. Patch tests were performed using ethchlorvynol on normal skin, and on a previously involved site. Erythema and oedema occurred on the latter area, but not on normal skin.

Dichloralphenazone.—Nearly half a million prescriptions each year are written for dichloralphenazone. This drug dissociates completely to its constituent moieties, phenazone and chloral hydrate (Trigger, 1967). Gomersall *et al.* (1966) reported an adverse reaction to dichloralphenazone. A woman aged 60 developed an erythematous rash with constitutional symptoms on 2 occasions after taking one tablet of this drug. Patch tests were strongly positive to dichloralphenazone and phenazone, but there was no reaction to chloral hydrate or saline. Fixed drug eruptions to phenazone have been reported more commonly than to chloral hydrate (McCullough and Zeligman, 1951), in fact, Brocq (1894) introduced the term fixed drug eruption to describe this special type of reaction to phenazone. Verbov (1972) reported a fixed eruption to dichloralphenazone, which was reproduced by a challenging dose of phenazone.

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John Almeyda
Ashley Levantine

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News and Notices

Psoriasis Research Award for 1971

The $1000 1971 Mr and Mrs J. N. Taub International Memorial Award for Psoriasis Research is given to Drs Kenneth M. Halprin, Phillip Frost and Gerald D. Weinstein of the University of Miami. These three individuals, sometimes working together and at other times independently, have each contributed significantly to our understanding of the pathophysiology of psoriasis.

The North American Clinical Dermatologic Society

The North American Clinical Dermatologic Society has arranged to meet in the Edward Lewis Theatre, Middlesex Hospital Medical School, London, W.1, on the mornings of May 15th, 16th, 17th and 19th, from 9.00 a.m. to 12.30 p.m.

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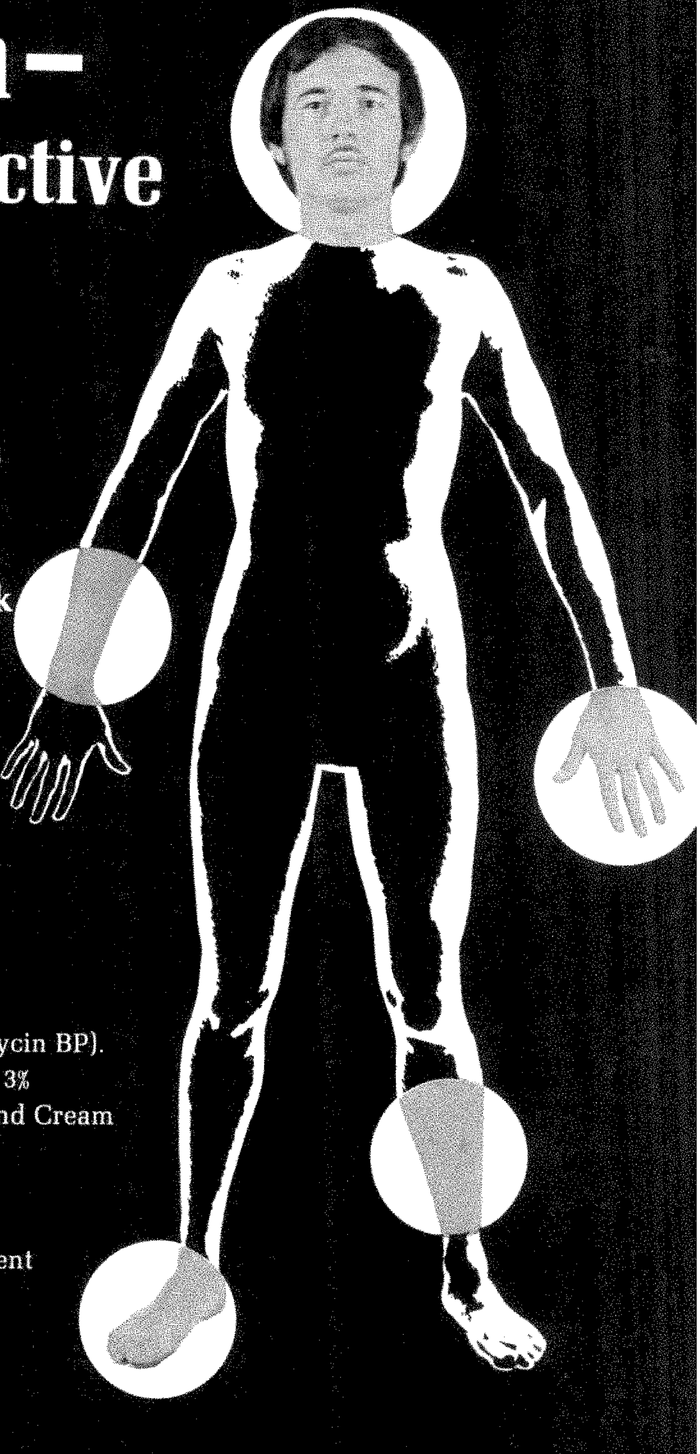

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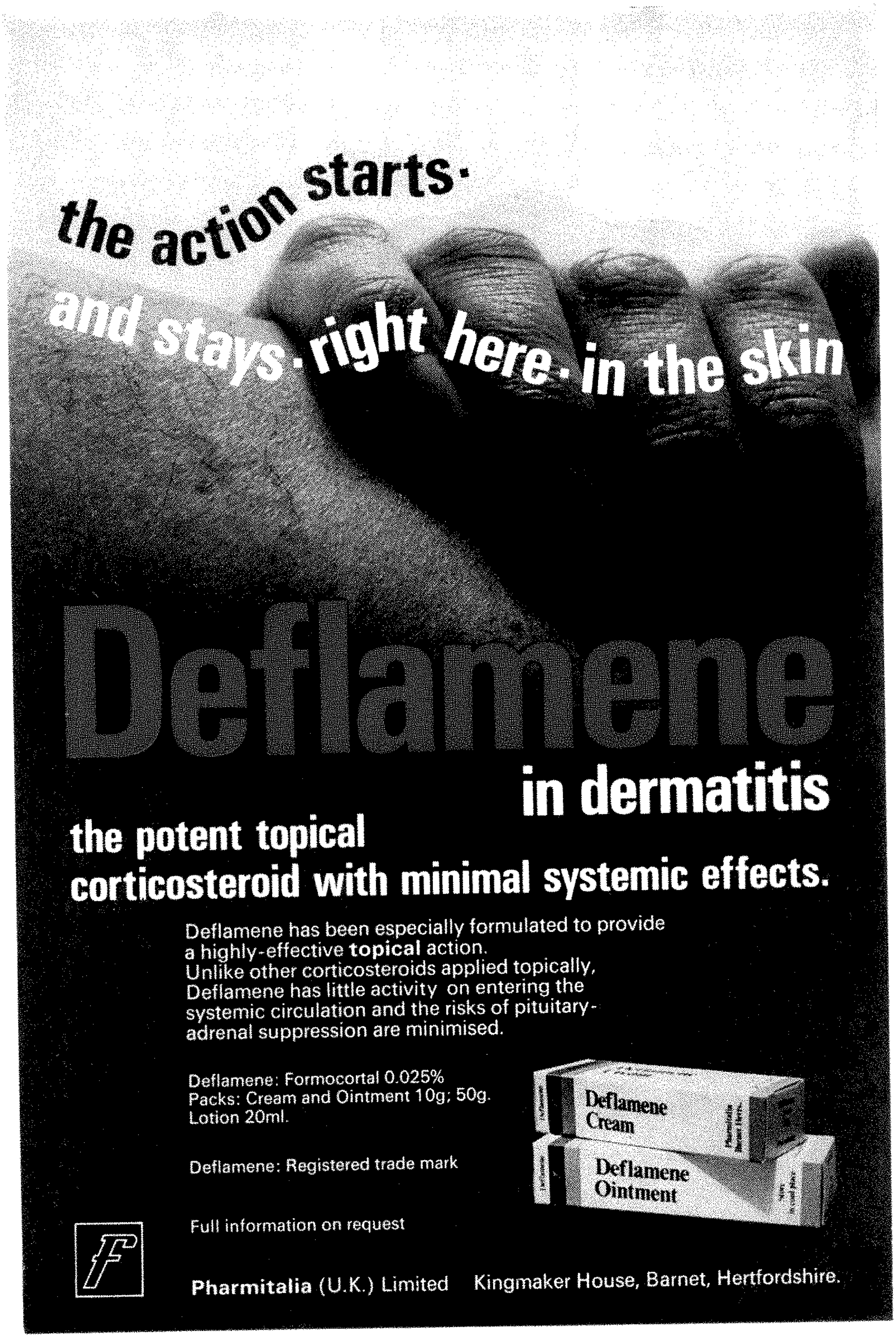

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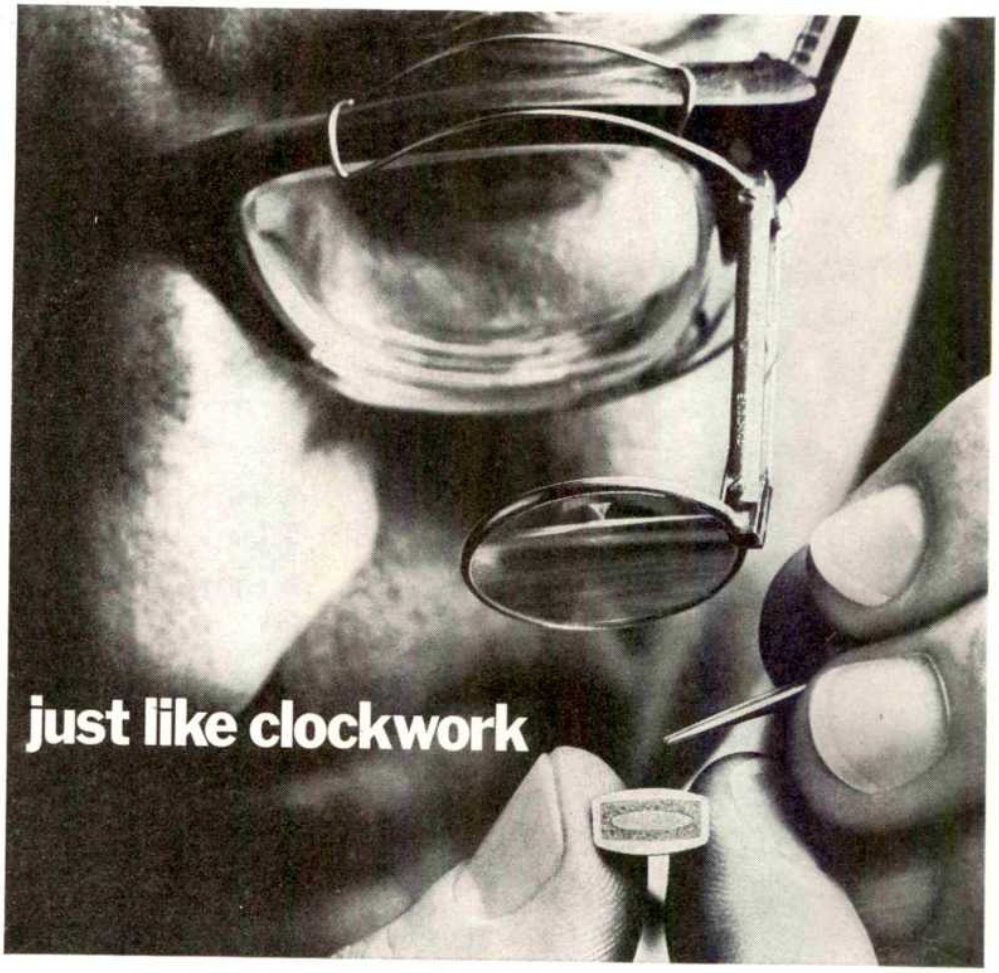

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CONTENTS (*continued*)

Books

A Treatise of Skin. Vol. 1—Biophysical Properties of the Skin (Elden (Edit.)); Cutane Paraneoplastische Syndrome (Herzberg (Edit.)); Acne Vulgaris (Frank) 308

Correspondence

" Metosyn " in Psoriasis and Eczema
B. PORTNOY and I. SARKANY 310
The Effect of a Medicated Wash on Acne, Sebum Excretion Rate and Skin Surface Lipid Composition
W. J. CUNLIFFE, J. A. COTTERILL and B. WILLIAMSON 311
Dapsone
J. O'D. ALEXANDER 312

Quarterly Review

Drug Reactions XVII. Cutaneous Reactions to Barbiturates, Chloral Hydrate and its Derivatives
J. ALMEYDA 313

News and Notices 316

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

Pityriasis Lichenoides: a Reappraisa.
R. MARKS, M. BLACK and E. WILSON JONES 215
Juvenile Dermatitis Herpetiformis in Jamaica: Clinical and Gastrointestinal Features
JOYCE WARNER, S. E. H. BROOKS, W. P. T. JAMES and SHEILAH LOUISY 226
Chronic Mucocutaneous Candidiasis, Endocrine Deficiency and Alopecia Areata
L. STANKLER and P. D. BEWSHER 238
Demonstration of Basal Cell Immunofluorescence in Drug-induced Toxic Epidermal Necrolysis
KENNETH M. STEIN, OTTO L. A. SCHLAPPNER, CHARLES L. HEATON and JAMES W. DECHERD 246
Rosacea-like Dermatitis: Contraindication or Intolerance Reaction to Strong Steroids
GERHARD WEBER 253
Skin Markings in Negro Children from the West Indies
DOROTHY I. VOLLUM 260
The Investigation of Photocontact Dermatitis in a Dye Manufacturing Process
J. S. GARDINER, A. DICKSON, T. M. MACLEOD and W. FRAIN-BELL 264
Scanning Electron Microscopy of the Stratum Corneum
RODNEY P. R. DAWBER, RONALD MARKS and J. ALAN SWIFT . 272
The Response of the Sweat Glands to β-Adrenergic Stimulation
J. A. WARNDORFF 282
A New Ultrastructural Finding in Oral Submucous Fibrosis
W. H. BINNIE and R. A. CAWSON 286

Pharmacology and Treatment

Out-Patient Treatment of Ichthyosis: A Double-blind Trial of Ointments
F. M. POPE, J. K. REES, R. S. WELLS and K. G. S. LEWIS . . 291

Case Report

Linear Melorheostotic Scleroderma
LAWRENCE T. WAGERS, ALEX W. YOUNG, Jr. and STEPHEN F. RYAN 297

Comment

The Pathogenesis of Lichen Planus
MARTIN M. BLACK 302

Toulouse 1970 306

(*Continued on* p. iii *of cover*)

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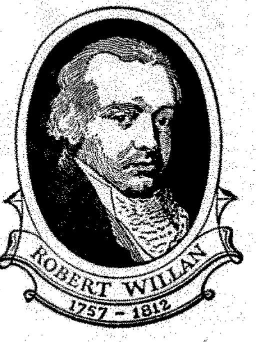

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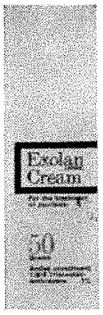

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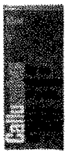

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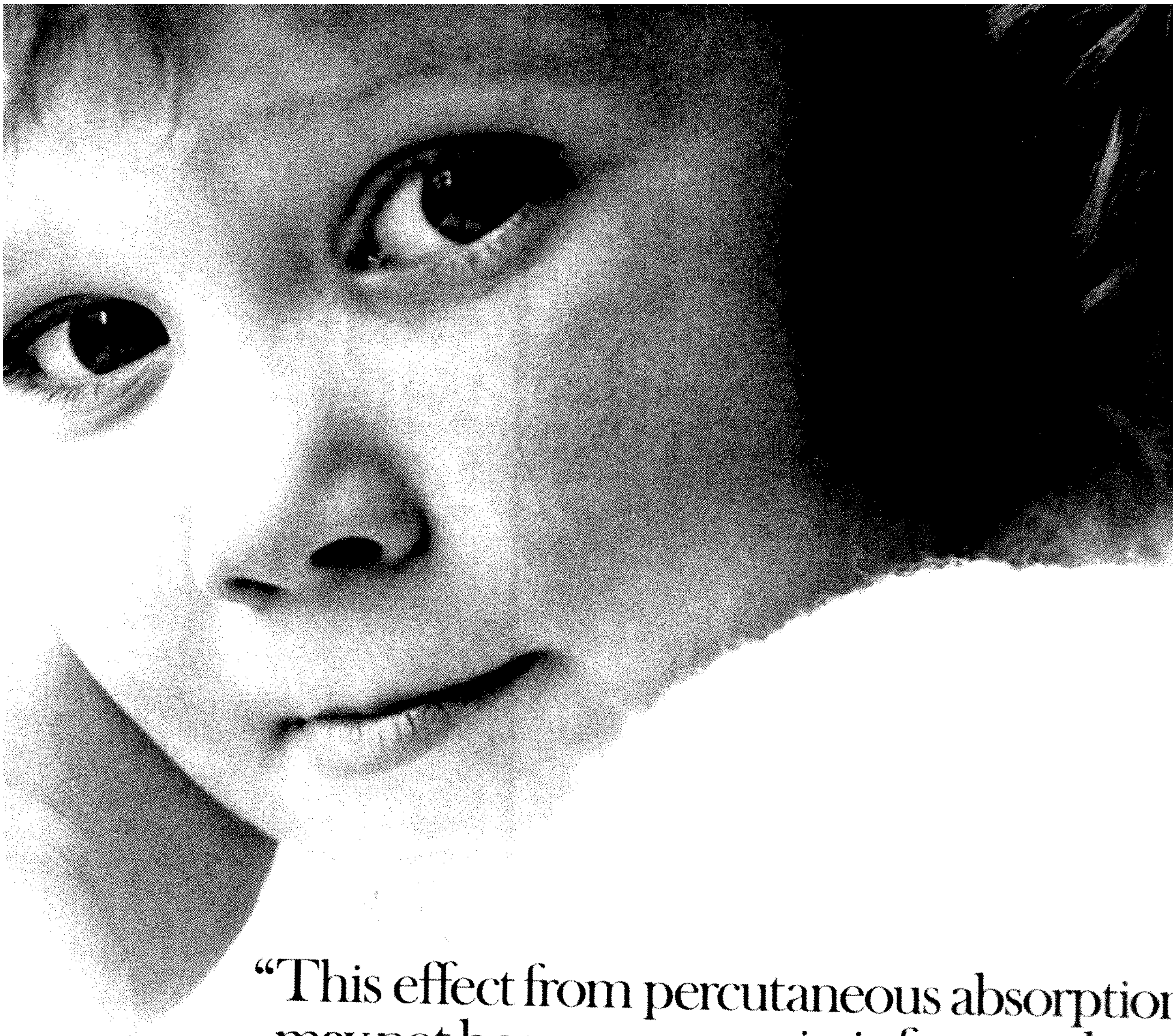

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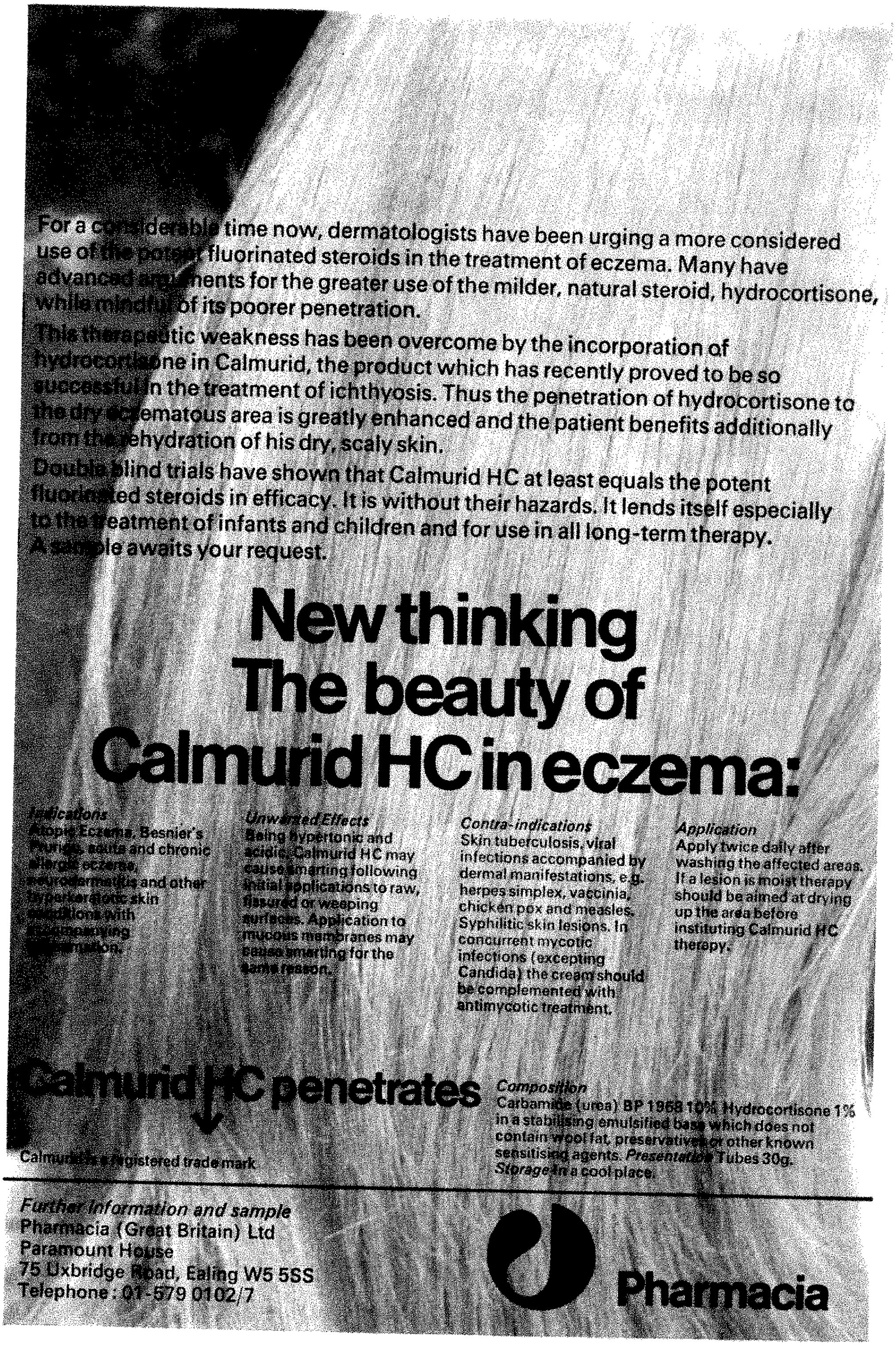

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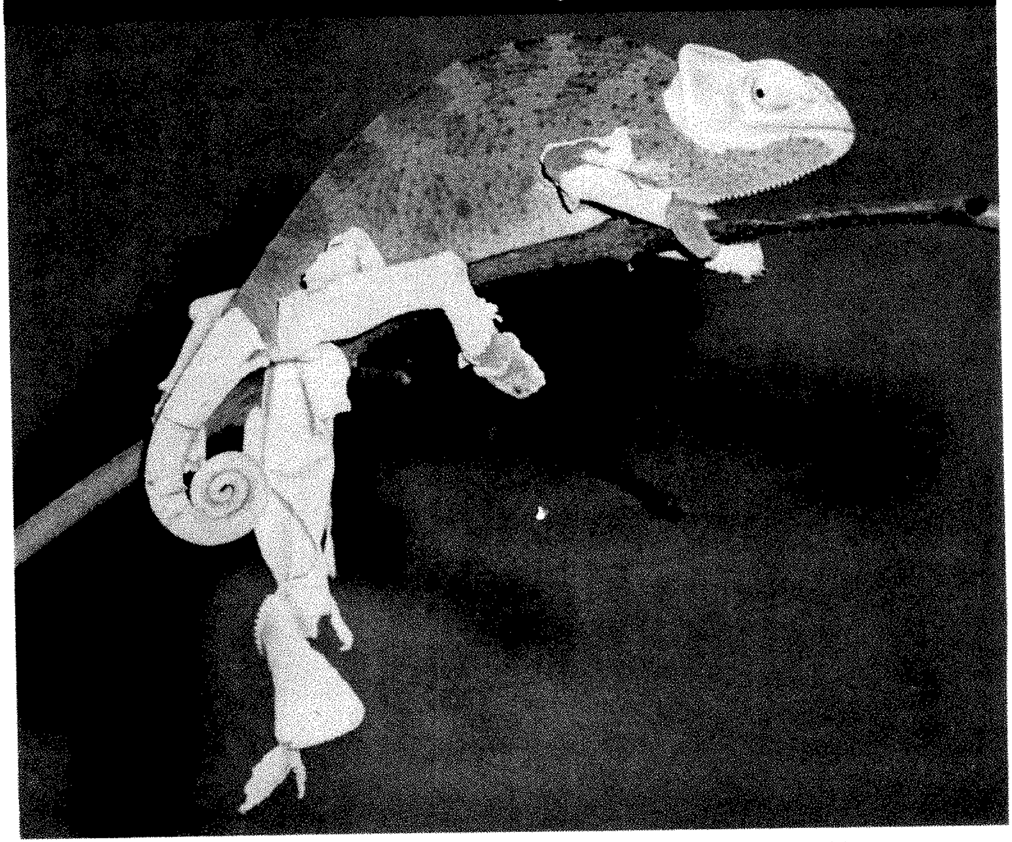

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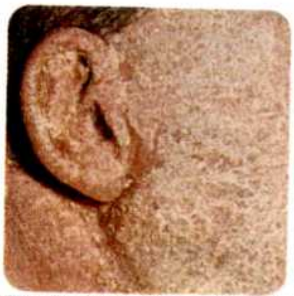
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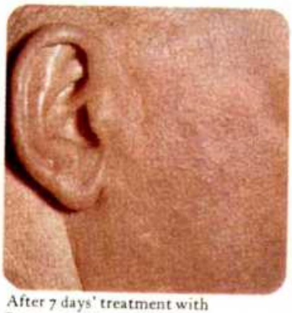
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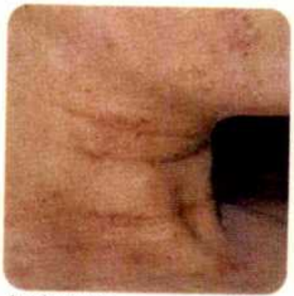
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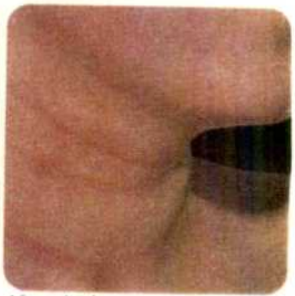
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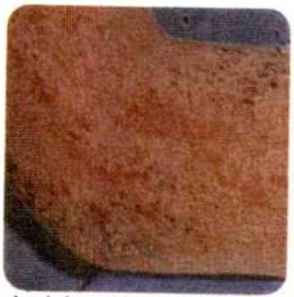
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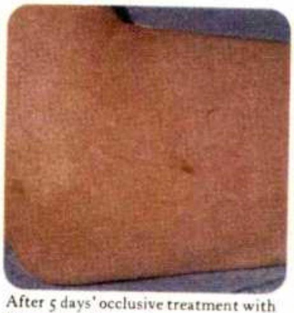
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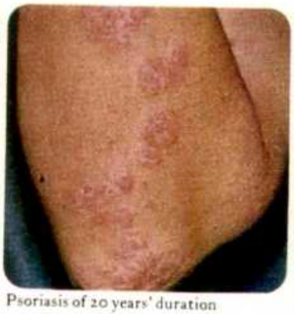
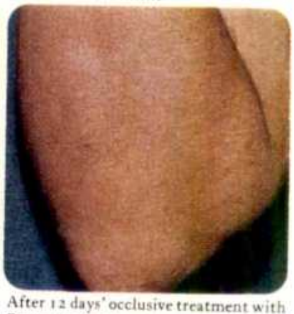
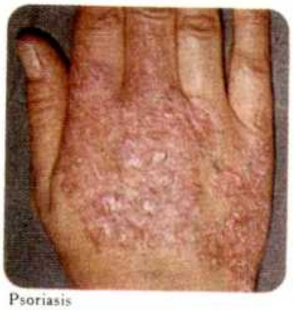
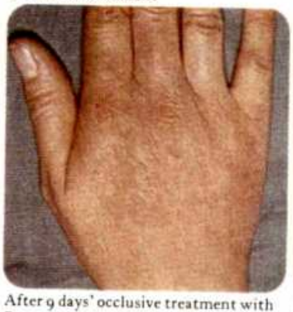
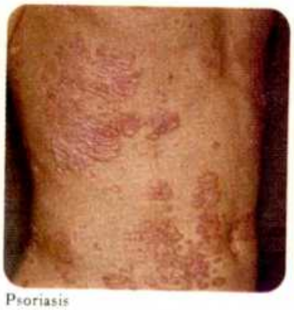
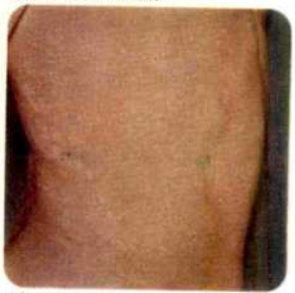

Psoriasis of 20 years' duration

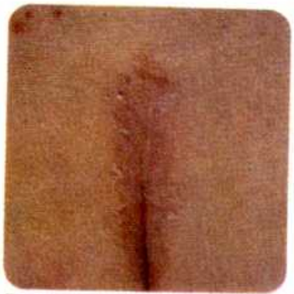

After 12 days' occlusive treatment with Betnovate Ointment

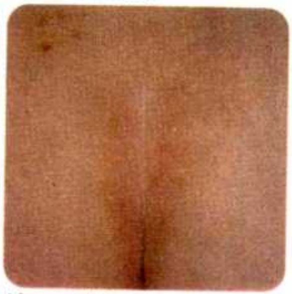

Psoriasis

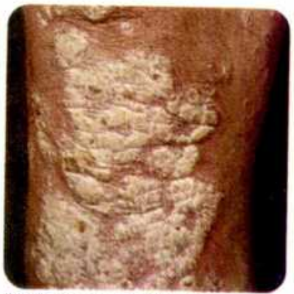

After 9 days' occlusive treatment with Betnovate Ointment

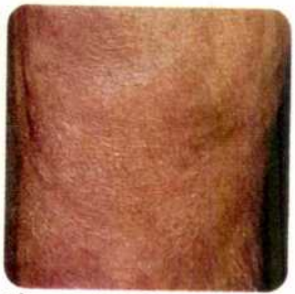

Psoriasis


After 21 days' occlusive treatment with Betnovate


Flexural psoriasis of 15 years' duration


After 4 weeks' treatment with Betnovate Ointment


Psoriasis of 51 years' duration


After 24 days' occlusive treatment with Betnovate Ointment

s the results.

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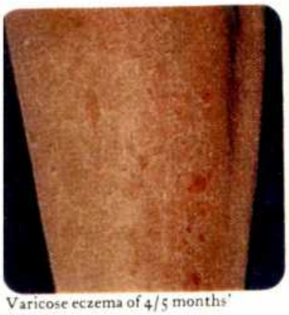
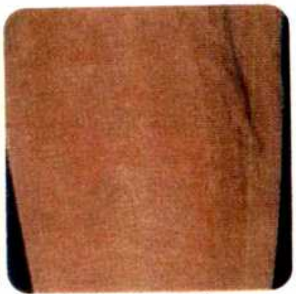
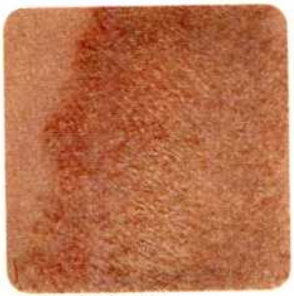
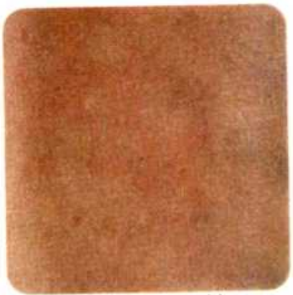
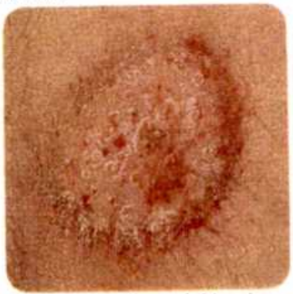

Varicose eczema of 4/5 months' duration

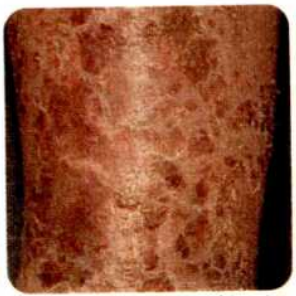

After 7 days' treatment with Betnovate Cream

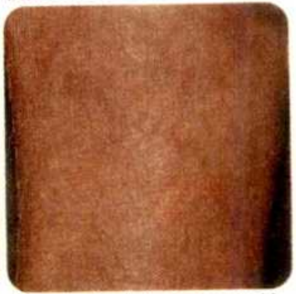

Constitutional eczema


After 4 weeks' treatment with Betnovate Cream


Discoid eczema of 9 months' duration

After 2 weeks' occlusive treatment with Betnovate Ointment


Atopic dermatitis of 12 years' duration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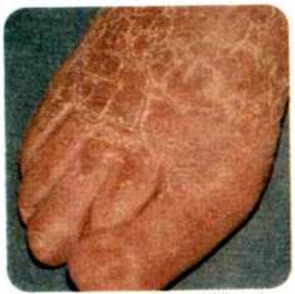
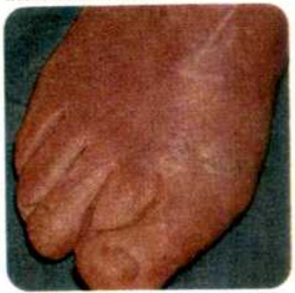

Contact dermatitis of 2 years' duration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*The Practitioner (1970) 205, 452-458.

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Clinical and Laboratory Investigations

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Department of Dermatology, University of Oregon Medical School, Portland, Oregon 97201, *U.S.A.*

SUPPRESSED CELL-MEDIATED IMMUNITY IN TWO ADULTS WITH ATOPIC DERMATITIS

WALTER C. LOBITZ, JR., JUAN F. HONEYMAN*
AND NORMAN W. WINKLER

Summary.—Tests of immune function over a 12-month period in a young woman and a young man with life-long atopic dermatitis showed depressed cell-mediated immunity (CMI) (delayed-type skin test unresponsiveness, inability to be sensitized to DNCB, diminished responsiveness of cultured lymphocytes to phytohaemagglutinin, enlarged subcutaneous lymph nodes without any cell-mediated immunopathology, but with the structure of a node involved in humoral antibody production). Direct immunofluorescence localized IgE in the germinal centres of these subcutaneous lymph nodes. No anti-immunoglobulin or anti-C_3 complement fluorescent conjugates were localized in their involved or uninvolved skin. Cells corresponding to small- and medium-sized lymphocytes in concentrated peripheral leucocyte smears stained with FITC antiserum to human IgE. The woman's lymphocyte transformation suppression improved during a remission and relapsed during an exacerbation of the dermatitis.

THE eczematous (atopic dermatitis) component of the state of atopy has always been difficult to explain. The immunopathogenic mechanisms, if any, to produce the eczema are still obscure. The weal and flare (Type I: Gell and Coombs) and reagin aspects of atopy, although reasonably understandable in the pathogenesis of hay fever, asthma and urticaria, do not seem directly responsible for the production of the eczema. On the other hand, the best understood eczematous disease, contact allergic eczematous dermatitis, is the result of a hyper-reactivity of cell-mediated immunity (CMI) (Type IV reaction: delayed hypersensitivity) specific to a contact allergen. But many patients with atopic dermatitis reflect a state of depressed CMI when they have a lowered resistance to herpes simplex and vaccinia virus (Turk, 1970). Larsen (1966) emphasized some of these paradoxical features of atopic dermatitis when he stated that eczema is classically thought to be related to the delayed hypersensitivity mechanism (Type IV reaction). However, the atopic type of eczema may occur in patients with Wiskott–Aldrich syndrome while they lack the ability to manifest delayed hypersensitivity (Aldrich *et al.*, 1954), indicating that the delayed hypersensitivity mechanism is not necessary in the genesis of the eczema. At that time (1966) circulating antibodies were thought not to be necessary either, since atopic dermatitis occurred in children with agammaglobulinaemia (Peterson *et al.*, 1962).

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* W. K. Kellogg Foundation Fellow (Dermatology) from the University of Chile Medical School.

We studied for over a year the clinical and immunological features of a young woman and a young man with life-long atopic dermatitis, searching for any associated immunopathology of atopic eczema.

THE PATIENTS

K.J., a woman aged 21, has had atopic dermatitis since the age of 1 year. Until she was 15 years old her atopic dermatitis involved the flexural aspects of her extremities and improved each summer. At the age of 15 the dermatitis became more generalized, sparing only her palms and soles, and no longer varied seasonally. Since childhood she has had chronic perennial hay fever, but no asthma. Exposure to wool, feathers, house dust and mould allergens was said to flare her rhinitis, but not her dermatitis. At the age of 10 systemic penicillin therapy produced generalized urticaria and laryngeal oedema. When she was 16 years old she had a severe attack of eczema herpeticum. Since then she has repeatedly developed recurrent herpes simplex labialis, all the while manifesting high titres of humoral antibodies to Herpes hominis type I virus. In her 21st year she had a unilateral ovariectomy for torsion of an ovarian cyst, and developed infectious mononucleosis. Since the age of 15 repeated patch tests to common contact allergens were never positive. Otherwise, her general health, physical examinations and abundant laboratory studies have not been unusual, except for a persistent peripheral blood eosinophilia that has ranged between 15% and 35%. Eosinophils were also abundant in her nasal mucosal smears and enlarged lymph node, but not in her skin. The immunological studies will be presented in table form.

P.H. is a 19 year old student and farmer who has had atopic dermatitis since infancy, perennial reactive airway disease (asthma) and allergic rhinitis since the age of 5, a generalized *Trichophyton rubrum* skin infection (*tinea cruris*, *manus*, *pedis*, *corporis* and *onychomycosis*), probably since his early teens, and recurring staphylococcal pyoderma. Although never completely free of flexural dermatitis, he did not develop generalized atopic eczema until the age of 17. His dermatitis is not seasonal, but he develops miliaria rubra in hot weather and urticaria in all flexural areas when he handles hay or farm animals, when he is sweating. For several years he was said to have weal and flare (Type I) dermal skin reactivity to antigens of horse, sheep, cattle, cat and dog hair, grass and weed pollens, inhalant moulds and house dust. Patch tests to common sensitizing antigens have always been negative. Completely eradicating his *Trichophyton rubrum* skin infection with oral griseofulvin therapy did not alter the course of his atopic dermatitis. Otherwise, his health, physical examination and abundant laboratory studies have not been unusual, except for a persistent blood eosinophilia that has been as high as 33%. The immunological studies will be presented in table form.

METHODS

1. *Intradermal skin tests* were performed with the following antigens: PPD (Tuberculin Purified Protein Derivative), stabilized solution (Connaught), intermediate strength (5 TU per dose); Histoplasmin (Parke-Davis Co.), dry powder (0·01 mg) dissolved in 1 ml buffered physiological saline; Coccidioidin (Cutter Labs.) 1 : 100 dilution; Mumps (Eli Lilly and Co.), undiluted; Dermatophytin (Hollister-Stier Labs.), 1 : 30 dilution; Dermatophytin " O " (Hollister-Stier Labs.) (oidiomycin: a Candidin), 1 : 100 dilution; purified glycoprotein antigens of *Trichophyton mentagrophytes* (GLYC/PROT 0·1 mg/ml pre and 0·05 mg/ml pre), *Trichophyton rubrum* (GLYC/PROT 0·1 mg/ml pre and 0·05 mg/ml pre) and *Candida albicans* (GLYC/PROT 0·1 mg/ml pre and 0·05 mg/ml pre) were made by us (Codner *et al.*, 1961); physiological saline (control). One-tenth ml of each solution was injected intradermally into the flexor surfaces of the arms. The injection sites were observed after 15 min for weal and flare (Type I) reactions, after 6 hours for the

Arthus response (Type III reactions), and after 2, 3 and 7 days for delayed (Type IV) reactions.

2. *DNCB* (*dinitrochlorobenzene*).—A 2% solution in absolute alcohol was applied to a 1·5 cm diameter area on the right flexor forearm to induce sensitization. After 2 weeks the DNCB challenge solution (0·1% in absolute alcohol) was applied to a similar area on the left flexor forearm. The sites were observed after 2, 7 and 14 days. All subsequent DNCB challenges were preceded by at least 2 weeks by re-sensitization. We were assured of the effectiveness of these tests since they were repeatedly positive when applied to other individuals used for controls.

3. *Immunodiffusion*.—Serum was studied by the radial immunodiffusion technique against standards (Crowle, 1961) for quantitative determination of IgG, IgA, IgM and C_3 complement.

4. *Immunofluorescence*.—Direct immunofluorescence studies were done on the involved and uninvolved skin of both patients, on the excised lymph nodes of patient K.J. and 3 normal (control) individuals, and on the smears of a suspension of peripheral leucocytes from both patients and 5 apparently normal persons, following the procedures of Friedman *et al.* (1960). Indirect immunofluorescence studies were done, using the sera of the 2 patients against normal human skin and monkey, cat and mouse oesophagus and liver. These procedures were performed according to Beutner *et al.* (1970). The blocking test was used as a control (Nairn, 1969). Goat fluorescein isothiocyanate-labelled antisera (Hyland Labs.), specific for human IgG (H and L chain), IgA (H chain), IgE (H chain) and complement C-3, demonstrated specific single band immunoelectrophoretic patterns against human sera. Characteristics of the conjugated antisera, as well as the antisera used for the blocking test, are summarized below.

TABLE I.—*Fluorescent Conjugates*

	Fluorescent conjugates						Control antiserum for blocking procedure	
Antiserum (goat) to human	Protein concentration (mg/ml)	FITC† concentration (mg/ml)	F/P‡ ratio (molar)	Precipitin titre (ring test)	Antibody§ concentration (mg/ml)	Dilution of conjugate used	Antibody§ concentration (mg/ml)	Dilution of antiserum used
IgG (H and L chain)	26·7	0·183	3·5	1 : 16	1·5	1 : 30	1·6	1 : 30
IgA (H chain)	55·0	0·492	3·6	1 : 8	1·0	1 : 10	1·06	1 : 10
IgM (H chain)	13·0	0·106	3·1	1 : 8	0·35	1 : 7	0·78	1 : 10
IgE (H chain)	23·2	0·140	1·5	NA*	NA*	1 : 10	NA*	1 : 10
C_3	26·5	0·114	1·7	1 : 64	2·7	1 : 50	1·95	1 : 40

* NA: not available.

† FITC: fluorescein isothiocyanate.

‡ F/P ratio: fluorescein concentration/total protein concentration.

§ Determined by reverse immunodiffusion assay for precipitating antibody.

The microscopy equipment consisted of a binocular Leitz "Ortholux" fluorescence microscope with a high pressure mercury arc lamp (HBO 200), a UG1 2 mm excitation filter and a K430 barrier filter. All the specimens were examined with a reflecting-type, dark-field condensor. Photomicrographs were taken with an "Orthomat" microscope camera using Anscochrome 500 colour slide film, daylight type (ASA 500, DIN 28). Control studies using skin and sera from patients who had such diseases as pemphigus, bullous pemphigoid, lupus erythematosus, etc., indicated that our techniques were satisfactory.

5. *Lymphocyte transformation test*.—The blood lymphocytes of both patients and non-atopic, age and sex matched normal persons were studied, using the procedures described by Bain (1970) and Hughes *et al.* (1970). Phytohaemagglutinin (Burroughs-Wellcome),

0·1 ml per ml of cell suspension, was added to Falcon plastic tubes containing a suspension of 2×10^6 lymphocytes in 2 ml of culture medium containing 81·5% TC 199 (Gibco), 16% foetal calf serum (Gibco), 0·04% L-glutamine (Hyland) and 40 units/ml penicillin and streptomycin (Gibco). The cells were harvested after incubation at 37°C for 3 days without agitation. The *in vitro* lymphocyte transformation was measured by the ^{3}H-thymidine (New England Nuclear Corp.) uptake calculated in disintegrations per min (DPM), using a series 720 liquid scintillation counter (Nuclear Chicago), and reported as a mean value of triplicate cultures. For this purpose 2 μCi of ^{3}H-thymidine were added to each tube 4 hours before harvesting. The 3 lymphocyte transformation tests were performed on patient K.J. at different stages of activity of her atopic dermatitis.

6. *Tissue immunocytology* (lymph nodes and skin).—Four-mm punch biopsy specimens were taken from involved and uninvolved skin. Lymph nodes were excised from the axilla of P.H., from the inguinal area of K.J. and from the inguinal areas of 3 apparently healthy, non-atopic individuals as controls for the direct immunofluorescence tests. Tissues were frozen immediately in liquid nitrogen and a portion also fixed in Helley's or in Carnoy's solution. These were stained with HE, methyl green pyronin, toluidine blue (pH 5) and for acid phosphatase.

RESULTS

Patient K.J.

Intradermal skin tests.—She had no immediate weal and flare (Type I), 6–12 h Arthus response (Type III), nor any delayed 48-h reactions (Type IV) when tested in August and October 1970, and February, May and June 1971, to PPD (tuberculin), histoplasmin, coccidioidin, mumps, dermatophytin or dermatophytin " O " (Candidin).

DNCB sensitization and challenge.—This was negative in September and November 1970, and in February, April and June 1971.

TABLE II.—*Quantitative Immunodiffusion (Serum)*

	mg/100 ml		
	August 13th, 1970	November 7th, 1970	Normal values
IgG	1125	1065	881–1519
IgA	148	208	167–408
IgM	95	110	51–109
C_3	140	160	123–167

TABLE III.—*Fluorescence Studies of Subcutaneous Lymph Nodes*

Fluorescent conjugates	Patient K.J.	3 normal persons
IgG	+++	+++
IgA	±	±
IgM	++	++
IgE	+++	Neg.
C_3	Neg.	Neg.

Indirect immunofluorescence tests.—The patient's serum was negative, using 4 separate fluorescent conjugates against 6 test tissues.

Direct immunofluorescence tests

1. *Skin biopsy specimens.*—Both involved and uninvolved skin were negative to all the fluorescent conjugates.

2. *Lymph nodes* (April 1971).—Fluorescence was observed only in the germinal centres (Fig. 1a).
3. *Peripheral blood cells* using FITC antiserum to Human IgE (the fluorescent cells corresponded to medium and small lymphocytes) (Ishizaka *et al.*, 1970; Ishizaka, 1970).

Tissue immunocytology.—(1) The skin. The epidermis had acanthosis with focal parakeratosis and focal spongiosis. There was a mild perivascular lymphocytic

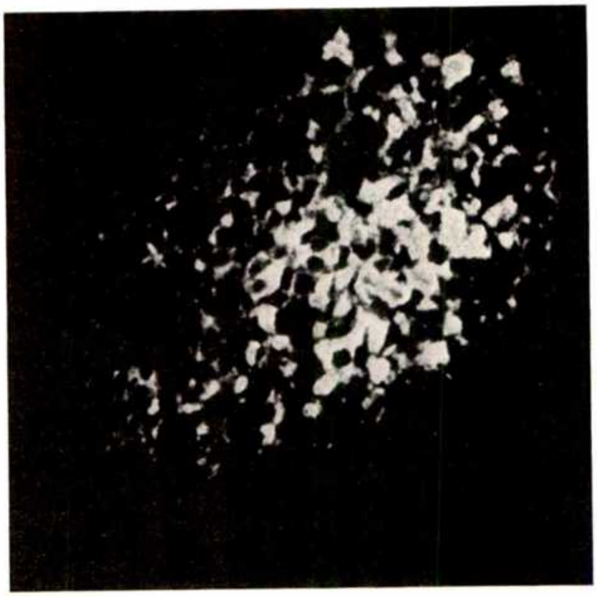


FIG. 1.—Direct immunofluorescence showing IgE localization in the germinal centre of K.J.'s subcutaneous lymph node (× 200).

TABLE IV.—*Peripheral Blood Cells Using Antiserum to Human IgE*

Subjects	% displaying anti-IgE fluorescence
K.J.	15
Normal 1	< 1
Normal 2	0
Normal 3	3
Normal 4	2
Normal 5	< 1

TABLE V.—*Lymphocyte Transformation Tests*

			Tritiated thymidine uptake (DPM)	
Person	Date	Activity of disease	No antigen	PHA
K.J.	10/30/70	4+	1182	22,959
Control	10/30/70	—	1255	62,828
K.J.	11/2/71	1+	2650	166,005*
Control	11/2/71	—	1912	86,320
K.J.	5/5/71	4+	1447	37,078
Control	5/5/71	—	1208	56,829

* Note that patient's disease was in remission.

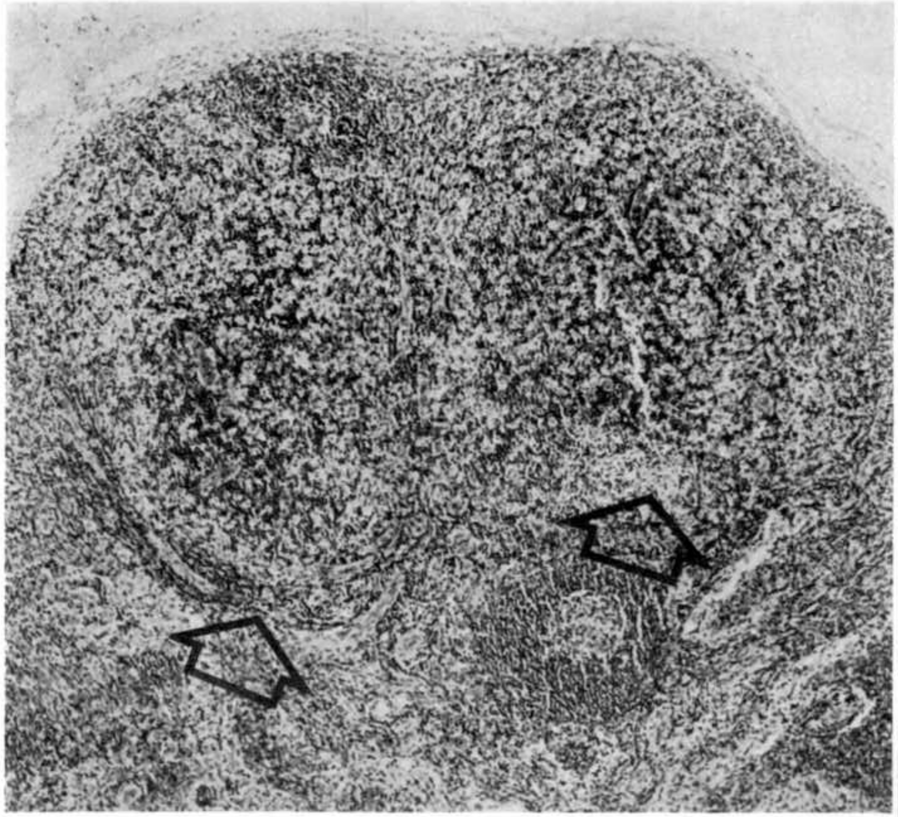


FIG. 2a.—Two active germinal centres of lymph node (arrows) (H. and E. × 200).

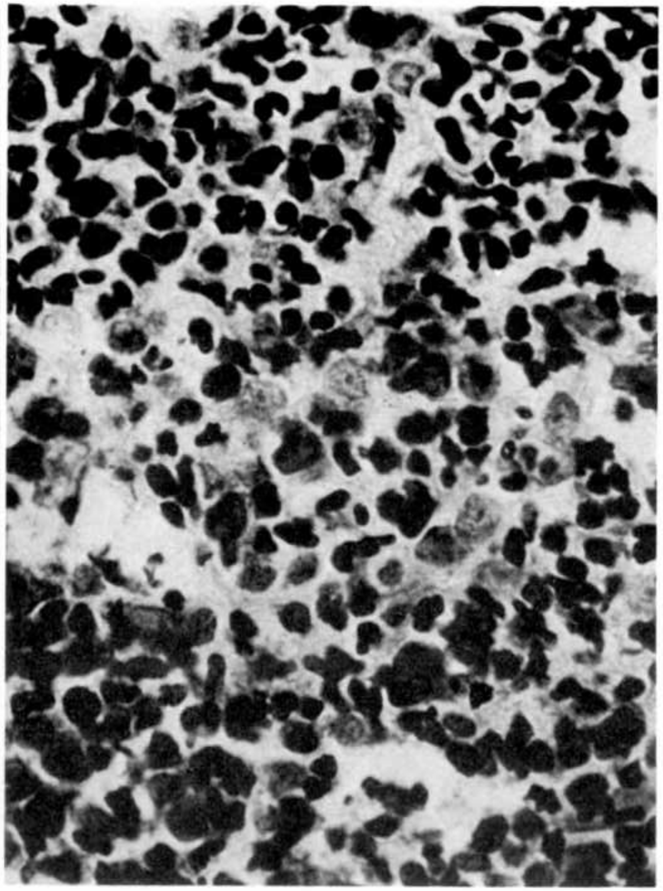
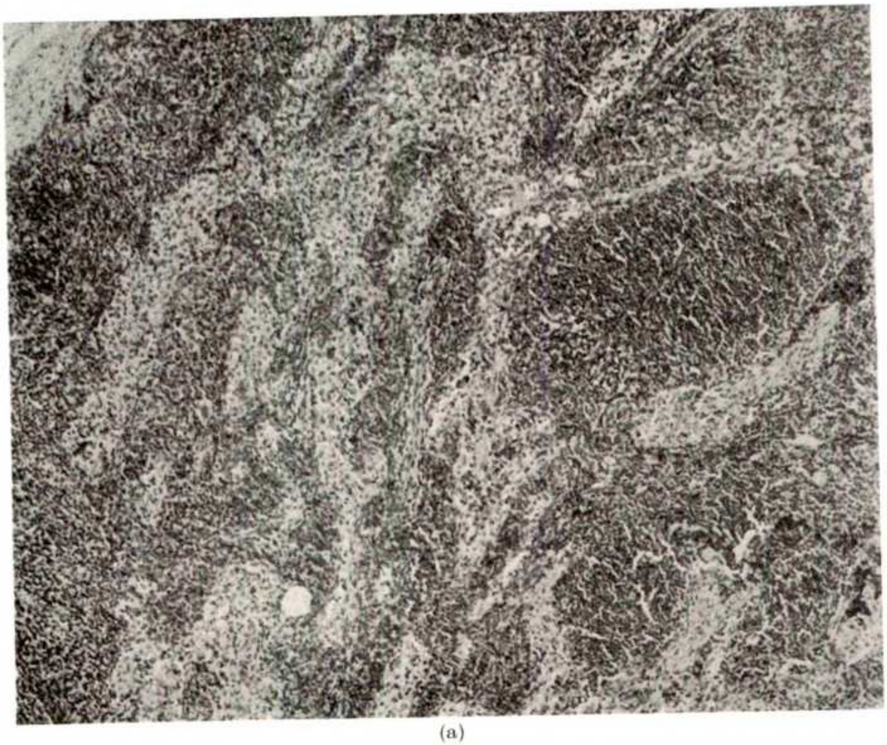


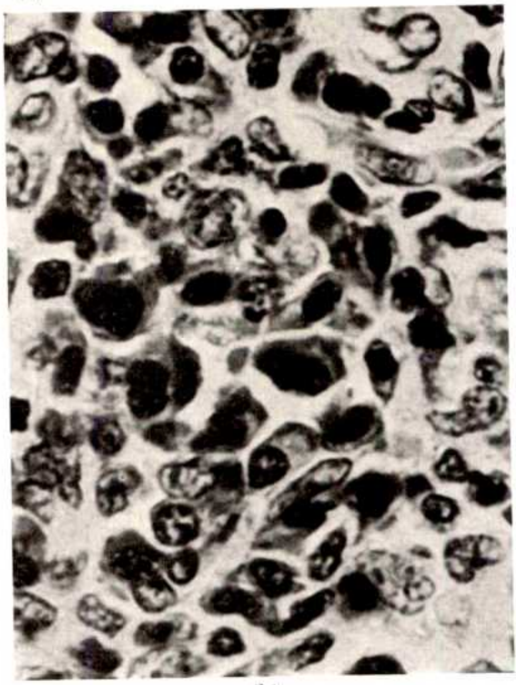
FIG. 2b.—Higher power of area in a germinal centre showing active immunoblasts (H. and E. × 400).



(a)

infiltrate. An occasional eosinophil was present. The cells in the dermis did not stain with methyl green pyronin or for acid phosphatase.

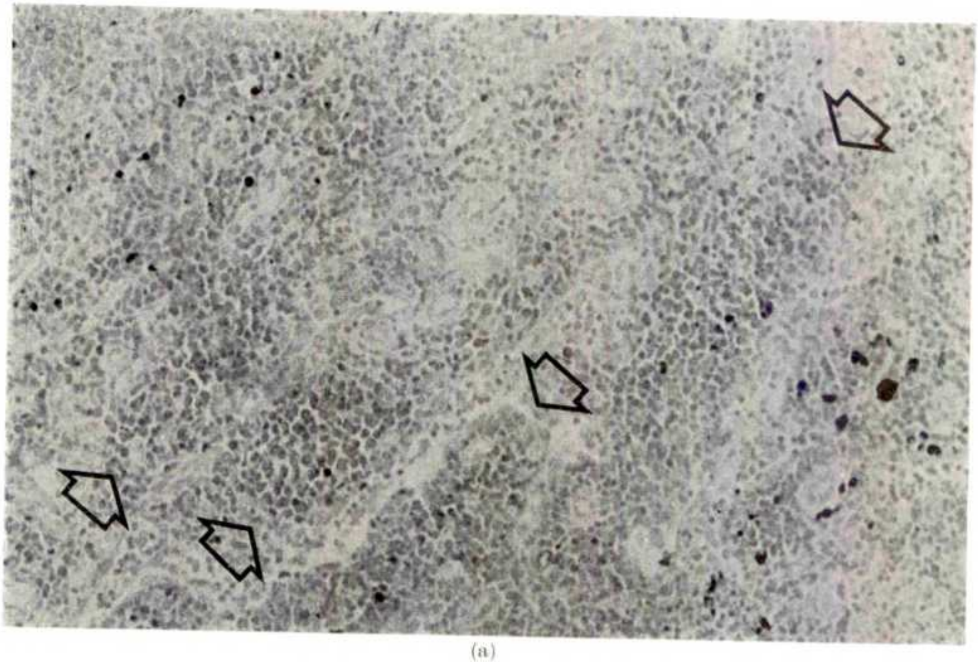
(2) The lymph node. The right inguinal node, histopathologically described as "dermatopathic lymphadenopathy" had the immunological features of a node involved exclusively in humoral antibody production (Turk, 1971). There was no evidence of any cell-mediated immune activity (Turk, 1971). The paracortical area was absent and germinal centres were large, containing many cells with much nuclear activity (Fig. 2a, b). Many cells stained with methyl green pyronin. The



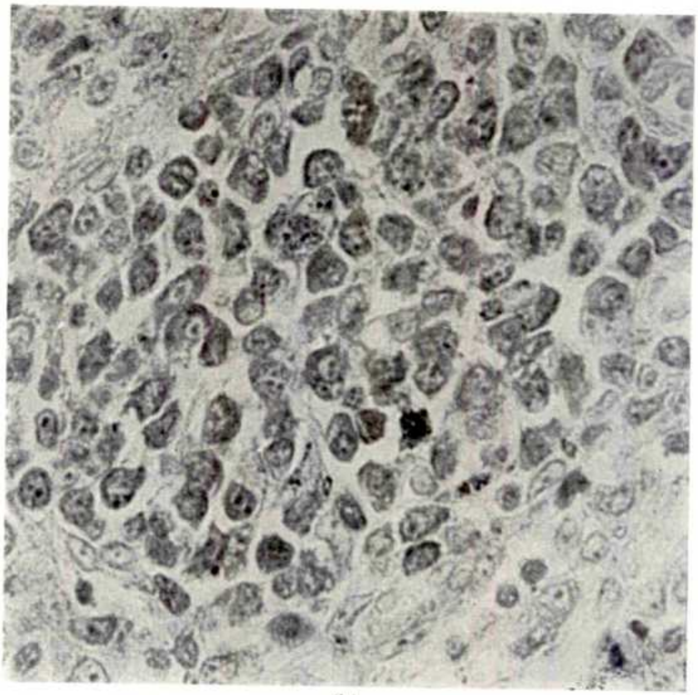
(b)

FIG. 3a.—Medulla and medullary cords of lymph node (H. and E. × 100).

FIG. 3b.—High power of medullary cord showing large plasmocytes and plasma cells (H. and E. × 400).



(a)



(b)

FIG. 4a.—Medullary cords (arrows) with pyroninophilic plasma cells (methyl green pyronin, × 100).

FIG. 4b.—High power of these pyroninophilic plasma cells (methyl green pyronin, × 400).

cortex had large masses of foamy histiocytes. The cortico-medullary junction and the medullary cords were rich in plasma cells and larger plasmocytes (Fig. 3a, b), which stained heavily with methyl green pyronin (Fig. 4a, b). Acid phosphatase positive macrophages were also present (Fig. 5). Numerous extravascular eosinophils were observed in the cortico-medullary junctions and medullary cords, admixed with plasma cells and small round cells.

Patient P.H.

Intradermal skin tests.—He had no positive delayed reactions (Type III or Type IV) to antigens of PPD (tuberculin), histoplasmin, coccidioidin, mumps, dermatophytin and dermatophytin " O " (Candidin) in June 1970 and June 1971.

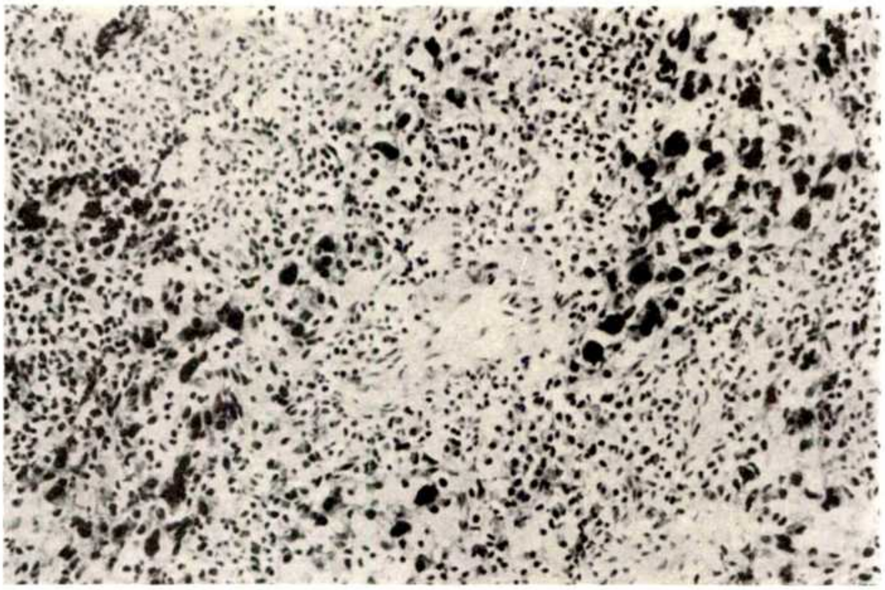


FIG. 5.—Dark staining acid-phosphatase positive macrophages in the medulla of the lymph node.

In June 1971 he had no delayed reactions (Type IV) to the purified glycoprotein antigens of *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Candida albicans*; but he had immediate weal and flare reactions (Type I) to both of the *Trichophytin* antigens and to the commercial Dermatophytin solution.

DNCB sensitization and challenge.—The tests were negative in September and November 1970, and February and April 1971.

Indirect immunofluorescence tests.—Same as K.J.

Direct immunofluorescence tests.—Skin and peripheral leucocytes the same as K.J. (No direct immunofluorescence was done on the lymph node).

Tissue immunocytology.—(1) The skin. Biopsy specimens showed the same changes as K.J.

(2) The lymph node. The immunocytological interpretation is the same as that for K.J. The node was completely surrounded by a fibrous capsule. Active germinal centres were present in the cortex, but they were over-shadowed by

nodular collections of foamy histiocytes located beneath the germinal centres and extending part way into the medulla. The histiocytes had a pale-staining cytoplasm, vesicular nuclei and light eosinophilic nucleoli. There were no lipid deposits in the cytoplasm, but granules of melanin pigment were seen. Admixed with the histiocytes was a smaller number of normal lymphocytes. A few eosinophils were scattered throughout the node. The medullary sinuses were dilated by an approximately equal number of lymphocytes and histiocytes; a few of the latter contained phagocytosed debris. There were many plasma cells and plasmocytes in the medullary cords.

TABLE VI.—*Quantitative Immunodiffusion (Serum)*

	mg/100 ml	
	December 22nd, 1970	Normal values
IgG	1950	881–1519
IgA	176	167–408
IgM	70	51–109
C_3	230	123–167

TABLE VII.—*Lymphocyte Transformation Test*

			Tritiated thymidine uptake (DPM)	
Person	Date	Activity of disease	No antigen	PHA
P.H.	6/22/71	4+	1883	2,817
Control	6/22/71	—	1768	79,136

DISCUSSION

A person's immunological responsiveness depends upon cell-mediated immunity and humoral antibody production. These immune systems may be normal, hyper-reactive or hypo-reactive. The hypo-reactivity may be antigen-specific or nonspecific. The cause of such variations are hereditary or acquired (primary or secondary) and may reside in the afferent phase (antigen recognition and processing) or the efferent phase (production of mediators and reactions in tissues) of the response, or both.

We studied 2 young adults with life-long atopic dermatitis, both of whom failed to display delayed dermal reactivity (Type IV) to the antigens. This lack of Type IV reactivity, the poor response of their cultured lymphocytes to a nonspecific mitogen (PHA) when their eczematous disease was active, and the lack of CMI activity in their enlarged subcutaneous lymph nodes leads us to consider that their CMI unresponsiveness was not specific to a single antigen. Although we did not investigate any mediators in the efferent phase of their CMI systems, the studies indicate that the unresponsiveness might be in the afferent phase. Despite many reports, not enough is known of the immunological status of persons with atopic dermatitis for it to be possible to say whether this CMI defect is primary or secondary. It seems likely that the CMI unresponsiveness in our patients must be incomplete, since they are otherwise reasonably healthy.

A normal CMI system is not necessary for the development of atopic dermatitis, since some patients with Wiskott–Aldrich syndrome lack delayed (Type IV) dermal reactivity; but is a depressed CMI system necessary for the development of atopic dermatitis? Is the CMI depression secondary to whatever is causing the eczematous component of atopy? Defects in CMI have been recognized previously in patients with atopic dermatitis (Kaufman *et al.*, 1970). Such patients frequently develop eczema herpeticum or eczema vaccinatum (Turk, 1970). Their delayed (Type IV) reactivity to skin tests with common mould, bacterial and viral antigens is less responsive than non-atopics (Rajka, 1963, 1970). Their cultured blood leucocytes tend to respond to PHA with fewer mitoses than normal controls (Fjelde *et al.*, 1967).

Interestingly, cell-mediated, antigen-specific hypersensitivity was demonstrated in hay fever subjects who did not have eczema (*in vivo* positive delayed (Type IV) skin tests, and *in vitro* lymphocyte transformation and positive leucocyte and macrophage migration inhibition tests) (Brostoff *et al.*, 1969).

Patients with atopic dermatitis are also known to have high serum levels of IgE (Ishizaka *et al.*, 1970; Ishizaka, 1970; Juhlin *et al.*, 1969; Johansson *et al.*, 1970; Ogawa *et al.*, 1971) which seem to correlate with the severity of the dermatitis. Yet it is not certain whether a high and constant production of the short-lived IgE (half-life of 2 days) is necessary to develop atopic dermatitis. It is also not known whether IgE plays any role in causing the eczema, since patients with only allergic rhinitis or asthma, but no eczema, also have high serum levels of IgE, as do non-atopic individuals infested with *Ascaris*. Also, many atopic patients have a normal serum level of IgE (Johansson, 1967).

Although we did not determine the IgE serum levels in our patients, we did demonstrate IgE localized in the germinal centres of K.J.'s subcutaneous lymph node at a time when she had no increase in the severity of her dermatitis and was free of allergic rhinitis. Ishizaka (1970) reported that the subcutaneous lymph nodes were the least likely to show IgE localization in germinal centres of all the lymphoid tissue that he had studied. However, such " dermatopathic lymphadenopathy " is a hallmark of chronic atopic dermatitis.

The final expression of a functioning immune system is not antibody production (Craddock *et al.*, 1971) but the cells and their mediator products generating the inflammation. The binding of reaginic immunoglobulins to human cells has been studied through the ability of the treated cells to release histamine and slow-reacting substances of anaphylaxis (SRS-A) (e.g. mast cells, neutrophils, lung tissue) (Ishizaka, 1970; Osler, 1970). But the " binding of reagins to cells lacking histamine (e.g. lymphocytes) is not registered in this reaction " (Osler, 1970). With direct immunofluorescence we demonstrated IgE attachment to the peripheral blood leucocytes of both of our patients. The majority of these cells were small and medium-sized lymphocytes. It therefore seems unlikely that they elaborated a vasoactive amine to inflame the skin. Does IgE fixation to lymphocytes release an eczema (spongiosis)-producing factor? What does such IgE fixation to the lymphocytes do to the CMI unresponsiveness in these patients?

Kaufman and Hobbs (1970), who reported immunoglobulin deficiencies in a large atopic population (7% of whom had deficiency of serum IgA; our 2 patients had normal serum IgA levels), offered an intriguing hypothesis " that atopy develops in individuals whose immunological vocabulary is backward." Certainly the atopic immunological vocabulary is different, but perhaps it is our immunophilology which is still too backward to translate the immunological vocabulary of atopic dermatitis into an understandable language.

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"LYMPHOMATOID" PITYRIASIS LICHENOIDES; A VARIANT WITH HISTOLOGICAL FEATURES SIMULATING A LYMPHOMA

A CLINICAL AND HISTOPATHOLOGICAL STUDY OF 15 CASES WITH DETAILS OF LONG TERM FOLLOW UP

MARTIN M. BLACK AND EDWARD WILSON JONES

Summary.—The clinical and histopathological features of 15 cases with "lymphomatoid" pityriasis lichenoides are described. The eruption was characterized by the continuous formation of papules which involuted spontaneously to leave scars. The papules closely resembled those found in typical pityriasis lichenoides acuta or chronica, but in some cases larger ulceronecrotic lesions also occurred. The disease had often persisted for many years without evidence of systemic involvement. In 1 male, however, the outcome was fatal after an interval of 16 years. The spectrum of histopathological findings varied considerably from a benign-looking infiltrate, usually in the smaller papules, to those which suggested a highly malignant infiltrate because of the presence of numerous large atypical cells. Preliminary histochemical investigations suggest that the large cells are derived from histiocytes and are distinct from leukaemic blast cells or Hodgkin's giant cells.

THE term "pseudomalignancy" has been used to denote tissue reactions in which the histopathology appears to indicate malignancy, whereas the clinical course of the lesion or condition is unequivocally benign. Juvenile melanoma and kerato-acanthoma are amongst the best known examples of pseudomalignant conditions in dermatology, but several other forms are known (Wilson Jones and Marks, 1970). In 1968 Macaulay described a single patient with a self-healing papular eruption of long duration associated with the histological features of a malignant lymphoma, for which he introduced the term: lymphomatoid papulosis. Clinically similar cases have been described by Kasumbeili (1960); Dupont (1965); Kopf and Andrade (1966); Verallo and Haserick (1966); Piñol Aguadé *et al.* (1967); Couperus (1967); Berardi *et al.* (1968); Borrie (1969); Kawada *et al.* (1969) and Muller and Schulze (1971), in which atypical histological features were present, suggestive of a lymphoma or mycosis fungoides. The skin lesions have been described as being similar to those found in pityriasis lichenoides, although any possible relationship between the 2 conditions has been disputed. In this presentation we describe the clinical and histopathological findings of 15 patients with what we consider to be an unusual variant of pityriasis lichenoides (Mucha-Habermann). Details of long term follow up are presented and the relationship of our cases to other forms of pityriasis lichenoides are illustrated and discussed.

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PATIENTS AND METHODS

For the purpose of this study we have selected our cases on the basis of the following clinical and histological features:

1. An eruption characterized by a benign clinical course with the formation of cutaneous papules, often for prolonged periods of time, which tend to undergo necrosis but later involute spontaneously to leave atrophic or varioliform scars;
2. The presence in the inflammatory infiltrate of large atypical cells in sufficiently large numbers which might suggest the diagnosis of a malignant lymphoma.

We have defined an atypical cell as one at least twice the size of a small lymphocyte which contains a large hyperchromatic nucleus. In each biopsy an approximate estimate of the proportion of atypical cells present in the inflammatory infiltrate was made and then graded as follows:

0	No atypical cells present
±	Comprising < 10% of the inflammatory infiltrate
+	Comprising 10–30% of the inflammatory infiltrate
++	Comprising 30–50% of the inflammatory infiltrate
+++	Comprising 50–80% of the inflammatory infiltrate
++++	Comprising > 80% of the inflammatory infiltrate

Our selection of cases was somewhat arbitrary, as small numbers of atypical cells (less than 10%) have been noted in typical pityriasis lichenoides (Marks and Wilson Jones, in preparation). In this series at least one of the biopsies contained approximately 10% (Grade +) or more of larger atypical cells.

Nine patients had attended St John's Hospital during the past 19 years. The clinical details and sections from 2 cases had been sent to the late Dr Henry Haber, and from a further 3 to Dr Wilson Jones by outside dermatologists. Dr G. B. Dowling drew our attention to a further example (Case 1) which he had observed many years previously (Dowling, 1933). The following particulars of each case were recorded: age of onset, sex, duration of symptoms and a detailed clinical description of the types of lesions. Follow-up data was obtained on all cases for a minimum period of 2 years since the onset of the eruption, with particular reference to the state of general health.

Twenty-eight skin biopsies had previously been fixed in formalin and embedded in paraffin. Histological sections were prepared in the usual way and then stained with H. and E. A number of representative sections were also stained with Schiff's periodic acid stain, before and after digestion with diastase, an orcein stain for demonstrating elastic fibres, toluidine blue for metachromasia, Perl's potassium ferrocyanide method for blood pigment and the Feulgen stain for DNA.

In Case 15 a further biopsy of a fresh papule was obtained and this was frozen immediately in liquid nitrogen prior to its use for histochemical purposes. The following histochemical techniques were used:

1. *Hydrolytic enzymes*: Non-specific esterase (Holt's indoxyl acetate method, 1954); acid phosphatase (Gomori's lead method, 1941).
2. *Respiratory enzymes*: Succinic dehydrogenase (MTT method of Pearse, 1960); nicotine adenine dinucleotide diaphorase (NAD diaphorase; MTT method of Pearse, 1960).

CLINICAL FEATURES

The main clinical details are summarized in Table I. Eleven of our 15 cases were males. The age of onset of the youngest patient was 13 years, the oldest 62 years, the average age of onset being 38 years.

The earliest skin lesion in all cases consisted of an erythematous dermal papule. In 10 patients these papules evolved into lesions which closely resembled those found in pityriasis lichenoides acuta or chronica (Fig. 1). Many of the papules (0·5–1 cm) became haemorrhagic and necrotic before resolving spontaneously within 2 or 3 weeks to leave superficial atrophic scars. In 8 cases larger ulcero-necrotic lesions (Fig. 2) occurred and these lesions tended to leave deep varioliform scars. In 5 cases the papules developed an adherent mica-like scale. In 2 patients a few hyperkeratotic haemorrhagic plaques (3–5 cm) developed; these lesions persisted longer than the ulcerated papules, but also healed with scarring.

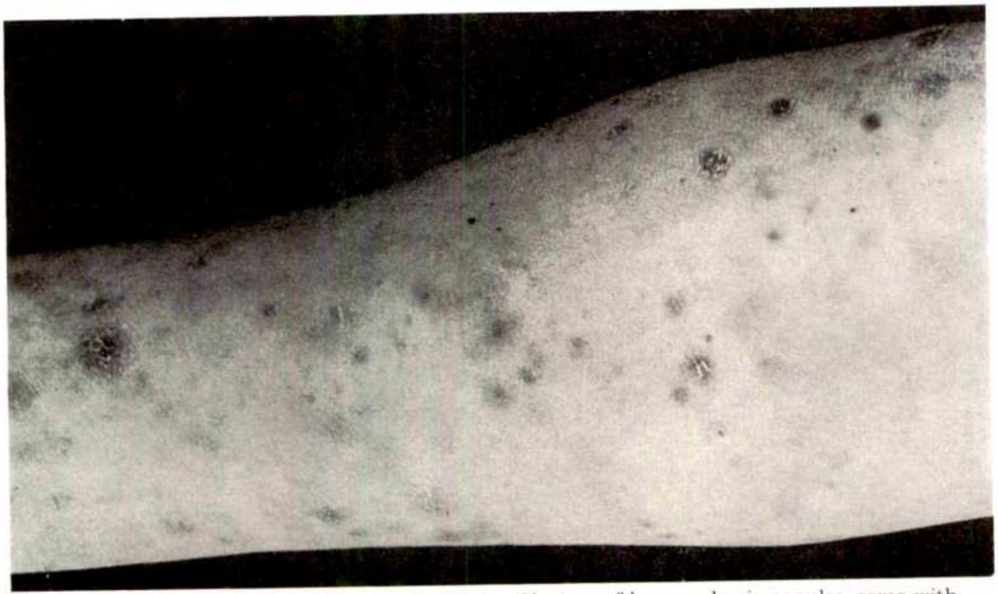

FIG. 1.—Case 12. Flexor aspect of forearm. Clusters of haemorrhagic papules, some with a mica-like scale. A lesion (2 cm) is situated near the volar aspect of the wrist.

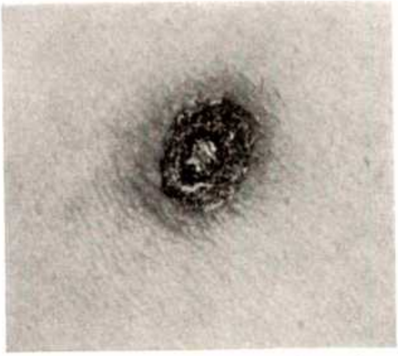

FIG. 2.—Case 7. Ulcerated papule (1–2 cm) on upper arm.

The predominant site to be affected was the trunk and limbs, but rarely lesions have occurred on the face, scalp, palms, soles and genitalia. General physical examination has revealed no abnormalities; in particular no lymphadenopathy or hepato-splenomegaly was found. Similarly, the haemoglobin, differential W.B.C. count and E.S.R. have been normal in all cases. No definite history of preceding illness or drug administration prior to the onset of the eruption was obtained. In 5 patients the eruption was of acute onset, with the development of about 50 to 100 papules. However, in the remaining 10 cases the onset was more gradual, with fewer lesions developing. In the majority the eruption of papules has not been associated with signs of systemic illness. In Case 3 a

TABLE I

Case	Sex	Age of onset	Duration (years)	Morphology of lesions	Distribution of lesions	Clinical course
*1	M	32	> 30	Papules (1–2 cm), becoming necrotic and healing to leave varioliform scars	Trunk, limbs, genitalia	Died, aged 89, from carcinoma of the lung, 57 years after onset of eruption
2	M	27	8	Small haemorrhagic papules (0·5 cm), some becoming necrotic and healing to leave atrophic scars	Trunk, scalp, face, limbs and genitalia	Alive and well 18 years after onset; no lesions
3	M	13	2–3	Small haemorrhagic papules (0·5–1 cm), some becoming necrotic and healing to leave atrophic scars	Trunk, limbs	Alive and well 8 years after onset; no lesions
†4	M	45	3 months	Small haemorrhagic papules (0·5 cm), the majority becoming necrotic and healing to leave atrophic scars	Trunk, limbs	Alive and well 10 years after onset; no lesions
5	F	47	13	Haemorrhagic papules (1–2 cm) some ulcerating and healing to leave varioliform scars	Trunk, limbs, scalp	Alive and well 13 years after onset; still getting the occasional new lesion
6	M	28	4	Small haemorrhagic papules, some with mica-like scale, others becoming necrotic and healing to leave atrophic scars	Trunk, limbs	Alive and well 8 years after onset; no lesions
7	M	62	8 months	Haemorrhagic papules (1 cm) the majority becoming necrotic and healing to leave varioliform scars	Trunk, limbs	Alive and well 2 years after onset; no lesions
8	M	41	3	Haemorrhagic papules (1 cm), the majority becoming necrotic and healing to leave atrophic scars	Trunk, limbs	Alive and well 6 years after onset; no lesions
‡9	M	20	4 months	Small haemorrhagic papules (0·5–1 cm), some with mica-like scale, the majority becoming necrotic and healing to leave atrophic scars	Trunk, limbs, face, scalp, soles and genitalia	Alive and well 2 years after onset; no lesions

10	M	29	28	Haemorrhagic papules (0·5–1 cm), ulcerating and healing to leave varioliform scars. Sometimes smaller papules with mica-like scale	Trunk, limbs	Alive and well 28 years after onset, but still getting the occasional new lesion
11	F	41	4	Small papules, with mica-like scale. Some become haemorrhagic and heal to leave atrophic scars	Trunk, limbs	Alive and well 4 years after onset, but still getting the occasional new lesion
12	F	35	5	Haemorrhagic papules (1–2 cm), becoming ulcerated and healing to leave varioliform scars. Smaller papules, with mica-like scale	Trunk, limbs, palms and soles	Alive and well 5 years after onset, but still getting new lesions
13	M	56	4 months	Haemorrhagic papules (1 cm), becoming necrotic and healing to leave atrophic scars. Smaller papules, with mica-like sale	Trunk, limbs	Alive and well 5 years after onset; no lesions
§14	F	25	7	Haemorrhagic papules (0·5–1 cm), becoming necrotic and healing to leave atrophic scars. A few atypical hyperkeratotic plaques (3–5 cm)	Trunk, limbs	Alive and well 7 years after onset, but still getting new lesions

* Shown at Clinical Meeting of Royal Society of Medicine (Dermatology Section) October 20th, 1932. Dowling, G. B. (1933) *Br. J. Derm.*, **45**, 34.

† Shown at Clinical Meeting of St John's Hospital Dermatological Society, December 1st, 1960. Wilson Jones, E. (1961) *Trans. a. Rep. St John's Hosp. Derm. Soc.*, **46**, 107.

‡ Shown at Clinical Meeting of St John's Hospital Dermatological Society, January 8th, 1970. Cream, J. J. (1970) *Trans. a. Rep. St John's Hosp. Derm. Soc.*, **56**, 76.

§ Shown at Clinical Meeting of Royal Society of Medicine (Dermatological Section) February 18th, 1971. MacMillan, D. C. (1971) *Br. J. Derm.*, **85**, 294.

TABLE II

Case	Clinical description of lesion biopsied	Proportion of atypical cells	Associated histological features
1	(a) Early papule	++	Epidermal oedema; early epidermal invasion
	(b) Ulcerated papule (2 cm)	+++	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage in upper dermis. Swelling of endothelium, with some fibrinoid deposition
2	Ulcerated papule (0·5 cm)	+++	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage in upper dermis. Swelling of endothelium. with some fibrinoid deposition
3	(a) Early papule with adherent scale	+	Moderate epidermal oedema and invasion. Vascular dilatation
	(b) Ulcerated papule (1 cm)	++	Marked epidermal invasion and necrosis. Vascular dilatation and swelling of endothelium
4	(a) Small established papule with adherent scale	+	Moderate epidermal oedema and invasion. Vascular dilatation and swelling of endothelium
	(b) Ulcerated papule (0·5 cm)	+++	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage. Some fibrinoid deposition
5	Early papule (1 cm)	+++	Moderate epidermal oedema and invasion. Vascular dilatation, with swelling of endothelium
6	Small established papule with adherent scale	++	Marked epidermal oedema and invasion. Vascular dilatation and swelling of endothelium. Occasional fibrinoid deposition
7	Early papule (1 cm)	++	Moderate epidermal oedema and invasion. Vascular dilatation and haemorrhage. Swelling of endothelium
8	Early papule (1 cm)	++	Moderate epidermal oedema and invasion. Vascular dilatation and swelling of endothelium
9	(a) Small established papule, with adherent scale	0	Moderate epidermal oedema and invasion Vascular dilatation
	(b) Small established papule, with adherent scale	0	Moderate epidermal oedema and invasion. Vascular dilatation
	(c) Ulcerated papule (1 cm)	++	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage. Swelling of endothelium
	(d) Ulcerated papule (0·5 cm)	+	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage. Swelling of endothelium

10	(a) Early papule (1 cm) 1956	++	Moderate epidermal oedema and invasion. Vascular dilatation and swelling of endothelium
	(b) Early papule (1 cm) 1971	±	Slight epidermal oedema and invasion. Vascular dilatation. Swelling of endothelium, with some fibrinoid deposition
	(c) Small established papule, with adherent scale (1967)	0	Moderate epidermal oedema and invasion. Vascular dilatation and haemorrhage. Swelling of endothelium
	(d) Small established papule, with adherent scale (1971)	0	Moderate epidermal oedema and invasion. Vascular dilatation and haemorrhage. Swelling of endothelium
11	Small established papule, with adherent scale	++	Marked epidermal oedema and invasion; early necrosis. Vascular dilatation and swelling of endothelium
12	(a) Ulcerated papule (2 cm) 1965	++	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage. Swelling of endothelium, occasional fibrinoid deposition
	(b) Ulcerated papule (1 cm) 1971	+	Marked epidermal invasion and necrosis. Vascular dilatation and swelling of endothelium
	(c) Small established papule, with adherent scale 1971	±	Moderate epidermal oedema and invasion. Vascular dilatation and swelling of endothelium
13	(a) Ulcerated papule (1 cm)	++	Marked epidermal invasion and necrosis. Vascular dilatation and haemorrhage. Swelling of endothelium
	(b) Established papule, with adherent scale	+	Marked epidermal oedema and invasion; early necrosis. Vascular dilatation and haemorrhage. Swelling of endothelium
14	(a) Established papule (1 cm)	++++	Moderate epidermal oedema and invasion. Infiltrate very dense and extending into subcutis, containing a few eosinophils. Vascular dilatation and swelling of endothelium
	(b) Resolving papule	0	Scarring throughout dermis
	(c) Hyperkeratotic plaque	++++	Only slight epidermal invasion. Dense infiltrate extending into subcutis, containing a few eosinophils

low grade pyrexia continued for 2 months after the onset of the eruption, and in Case 10 an episode of abdominal pain occurred for a few days, but without definite physical signs or evidence of occult bleeding.

In this study, which has been largely retrospective, it has not always been possible to get detailed information on the course of the disease in all the patients, but we have been able to obtain follow up information concerning the state of general health on all cases for a minimum period of 2 years since the onset of the eruption. Case 1 is of special interest because it is known that, despite the presence of numerous atypical cells in the inflammatory infiltrate, the patient lived in good health for 57 years from the onset of the eruption, and then died from an unrelated condition in old age. The shortest duration of symptoms was 3 months, and the longest for more than 30 years. In 7 cases the eruption continued for more than 5 years, and in 4 others for more than 10 years. Despite the prolonged course of the disease, all except 1 have remained well throughout. We now report the clinical details of a single patient who appeared similar in many respects, but where the outcome was eventually fatal.

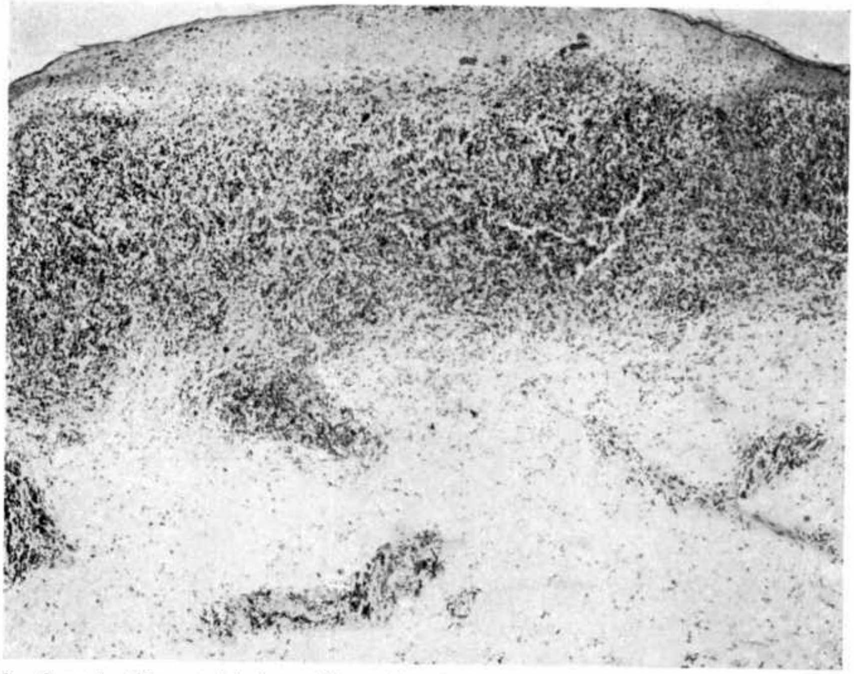

FIG. 3.—Case 2. Ulcerated lesion. The epidermis is necrotic over the centre of the papule. A dense infiltrate is present immediately below the necrotic epidermis, but also extends more deeply around small blood vessels. H. and. E × 25.

This patient will be reported in detail elsewhere (Samman, in preparation).

Case Report

A man aged 59 presented in 1964 with a 16-year history of a continuously cropping, widely disseminated eruption consisting of papulonecrotic lesions, many of which healed to leave prominent varioliform scars. An original biopsy revealed an intense wedge-shaped dermal infiltrate which contained numerous atypical cells (+++). The epidermis showed oedema and incipient necrosis, and was invaded by such cells. The patient was considered to have pityriasis lichenoides acuta with unusual "pseudoleukaemic" histological features (Samman, 1964). During the next 2 years larger haemorrhagic plaque-like lesions developed, many of which were slow to heal. A further biopsy of such a lesion showed a higher proportion (++++) of bizarre hyperchromatic cells, and mitoses were frequent. In 1966 the patient presented elsewhere with a large ulcerating mass on the anterior chest wall. He was anaemic and had enlarged firm lymph nodes in both axillae and in the neck, but the liver and spleen were not enlarged. Biopsy of the ulcerated mass was interpreted as an anaplastic sarcoma. He was treated with blood transfusions and local radiotherapy. The tumour mass regressed, but the patient died at home 6 months later. Unfortunately, no autopsy could be obtained.

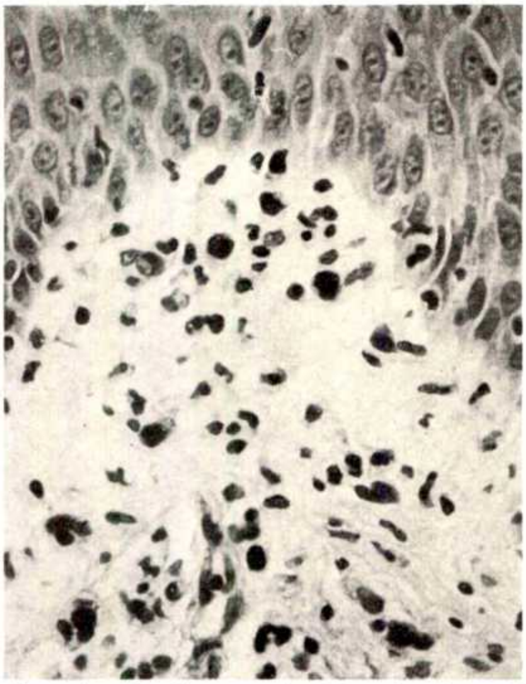

FIG. 4.—Case 4. Small papule with adherent scale. There is oedema of the lower epidermis and dermal papillae. The infiltrate contains a few large atypical mononuclear cells (Grade +). H. and E. × 250.

Histopathology

Details of the type of lesion biopsied, the percentage of atypical cells and certain other histological details are given in Table II. As can be seen, there was considerable variation in the histopathology according to the size, stage and type of lesion that had been selected for biopsy. The main histological feature common to most of the specimens was the presence of a dense abnormal lympho-mononuclear cell infiltrate in the dermis, associated with a variable degree of epidermal oedema and disruption.

The infiltrate.—In the great majority of specimens the infiltrate was most intense in the immediate subepidermal zone, with mononuclear cells forming a dense band below the epidermis (Fig. 3). At the margins of the lesion and towards the deeper parts of the dermis the infiltrate was mainly peri-vascular in arrangement.

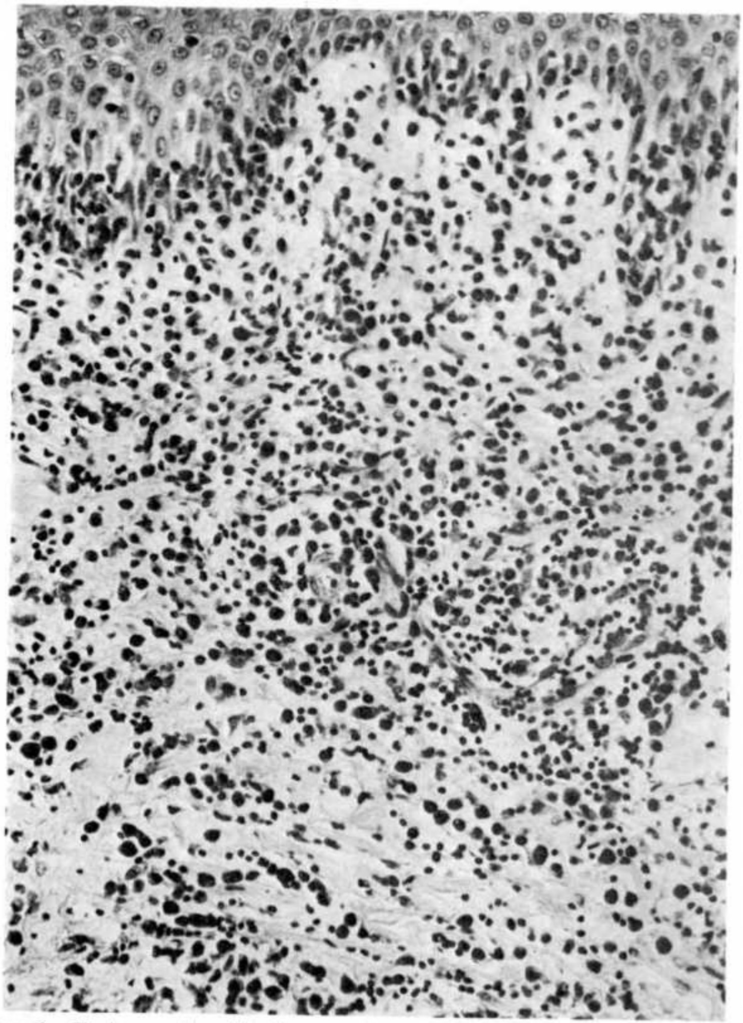

FIG. 5.—Case 5. Early papule. The lower epidermis is being invaded by both small mononuclear and larger atypical cells. Larger atypical mononuclear cells are prominent in the inflammatory infiltrate (Grade + + +). H. and E. × 160.

In some of the larger papules a wedge-shaped area of infiltrate extended deeply to reach the lower dermis or even the hypo-dermis in a few instances. In general, the infiltrate was densest towards the centre and sparsest at the periphery of the lesions. In at least 1 specimen from each patient the infiltrate contained large atypical cells possessing prominent hyperchromatic nuclei.

Such atypical cells were usually most numerous in the dermal papillae (Fig. 4) and around the upper dermal vessels towards the centre of the papules. In many specimens atypical cells were infiltrating the epidermis, particularly in its lower half (Fig. 5). The proportion of atypical cells in the infiltrate, as well as the size and degree of pleomorphism of the atypical cells, varied considerably (see Table II and Fig. 4, 5, 6). In some specimens, particularly in resolving lesions and those showing a mica-like scale, no atypical cells could be detected.

The cytology of the atypical cells.—The atypical cells varied greatly in size (15–30 μm) and morphology. A large deeply-stained nucleus usually occupied the greater part of such cells (Fig. 7). The amount of cytoplasm varied considerably, and in some cases it was reduced to a thin perinuclear rim. Usually the cytoplasm was well stained and its margins well defined. Under higher magnification it was apparent that the cytoplasm was finely granular, and in some cells a variable number of small microvesicles or clear spaces was noted.

In the majority of the specimens the nuclear details were obscured because the cells were so hyperchromatic. When lightly-stained specimens were prepared, it became apparent that the nucleus had a very irregular border due to folding and deep indentations of the nuclear membrane (Fig. 8). The chromatin was arranged in coarse overlapping granules. As a rule the nucleoli were not prominent, and they could be detected with ease only in understained sections. Mitotic figures were frequent, especially so in the cases with a high proportion of atypical cells. Sometimes cells with lobulated or kidney-shaped nuclei were seen as well as bizarre multi-nucleated forms.

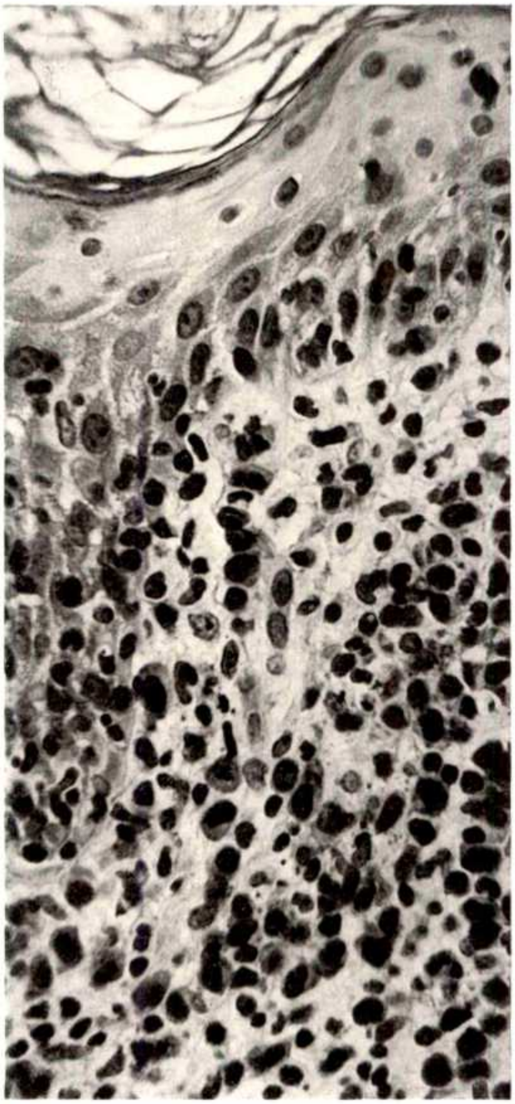

FIG. 6.—Case 2. Edge of ulcerated lesion. There is a dense infiltrate in the upper dermis, consisting predominantly of large atypical mononuclear cells (Grade +++). H. and E. × 250.

Apart from the frankly abnormal cells already described, the infiltrate was predominantly lymphocytic in nature. Besides small lymphocytes, cells intermediate in character between the atypical cells and small lymphocytes were seen. Such cells were similar in size to large lymphocytes, but the nuclei were heavily stained. The infiltrate usually also contained macrophages, and other histiocytic cells with a few mast cells. Polymorphonuclear cells, eosinophils and plasma cells were sparse or absent. Particularly in the vicinity of lesions showing central necrosis, mononuclear cells showing karyorrhexis were found.

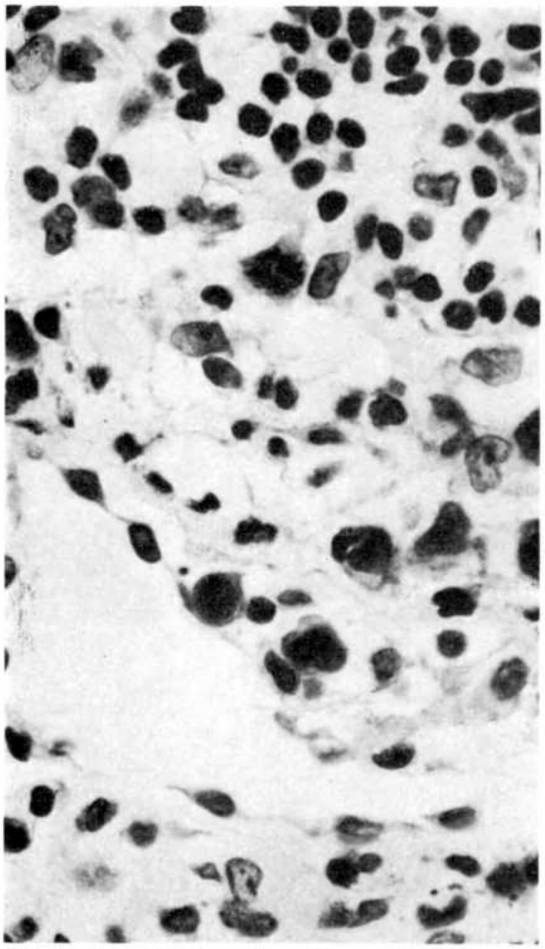

FIG. 7.—Case 2. A number of large atypical mononuclear cells are situated near a dilated capillary. These cells are at least 3 times the size of small lymphocytes (top half of the field) and contain a large deeply-stained nucleus. One is undergoing mitosis. H. and E. × 540.

Epidermis.—The degree of epidermal involvement and damage varied considerably with the stage and intensity of the disease process. Thus in early lesions the epidermis was intact apart from the presence of a few mononuclear cells penetrating into the basal cell layer. Conversely, in late necrotic lesions total destruction of the epidermis with ulceration was found over the top of the papules. In lesions of intermediate intensity a variable degree of oedema, vesiculation, and patchy individual cell necrosis occurred. In such lesions the stratum corneum was usually parakeratotic and was studded with micro-abscesses containing pyknotic mononuclear cell remnants. These micro-abscesses tended to be diffusely scattered in the parakeratotic layer rather than being aggregated into discrete foci as in psoriasis (Fig. 9). Invasion of the epidermis, whether damaged or not, by atypical and other mononuclear cells was a prominent feature in many of the specimens (Fig. 5, 9).

Blood vessels.—Small blood vessels in the upper and mid-dermis were invariably dilated and congested, and usually associated with oedema of the dermal papillae. Haemorrhage was prominent in 12 cases, and in some instances red blood cells as well as mononuclear cells had penetrated into the oedematous, or partially necrotic, epidermis above.

In areas of intense infiltrate some vessels appeared occluded due to endothelial swelling or deposition of fibrinoid material in the lesion. In 7 cases fibrinoid deposition in and around the vessel was prominent, but was not usually associated with vessel wall necrosis or with leucocytoclasis. Necrotizing vascular changes in all specimens except one were found only in the close vicinity of necrotic areas.

Special Stains and Histochemistry

No special features were detected with the metachromatic stains. Oedematous lesions did not show any marked increase in metachromatic staining, suggesting that accumulation of mucin was not a feature.

The PAS stain showed some increase in glycogen in the epidermis above non-ulcerated lesions. Some of the large atypical cells contained small PAS-positive granules in their cytoplasm. The granules could in part be removed by prior diastase digestion, suggesting the presence of glycogen. However, the majority of the atypical cells did not contain glycogen.

Feulgen stain.—This stain confirmed the high DNA content of the nuclei and was also helpful in outlining the morphological details. The coarse granular arrangement of the chromatin, as well as the nuclear indentation, was emphasized.

Histochemistry.—1. *Respiratory enzymes*: (Succinic dehydrogenase; NAD diaphorase). Respiratory enzyme activity was decreased in the epidermis over the centre of the papule. This part of the epidermis was oedematous and was beginning to undergo necrosis. The dermal infiltrate showed considerable respiratory enzyme activity.

2. *Hydrolytic enzymes*: (Acid phosphatase; non-specific esterase). The cytoplasm of the larger atypical cells showed marked hydrolytic enzyme activity and contrasted with lymphocytes or smaller mononuclear cells, in which the enzyme activity was weak or absent.

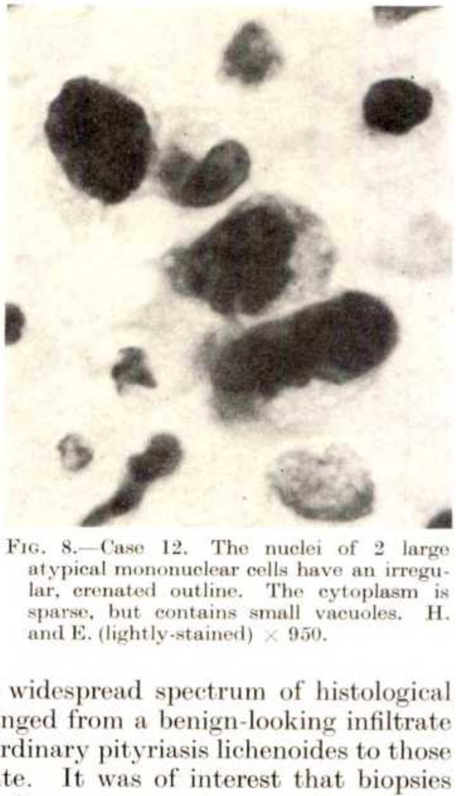

FIG. 8.—Case 12. The nuclei of 2 large atypical mononuclear cells have an irregular, crenated outline. The cytoplasm is sparse, but contains small vacuoles. H. and E. (lightly-stained) × 950.

Comments on the Histological Features

This study has clearly demonstrated the widespread spectrum of histological findings in this group of patients. These ranged from a benign-looking infiltrate in lesions that otherwise resembled those of ordinary pityriasis lichenoides to those which suggested a highly malignant infiltrate. It was of interest that biopsies taken at a later stage in the evolution of the disease in some instances looked less alarming than the initial biopsies.

Our definition of an atypical cell is somewhat arbitrary, as in reality every variation in cell size occurred between typical small lymphocytes and very large bizarre cells. The largest of the bizarre cells were similar in size to Sternberg Reed cells of Hodgkin's disease. However, most of the atypical cells found in the patients described here were different in that their nuclei were more hyperchromatic, the cytoplasm was less prominent and the cells lacked the large eosinophilic nucleolus characteristic of Hodgkin's cells. Nevertheless, a few of the atypical cells showed relatively large nucleoli, and the occasional cell was close in morphology to Hodgkin's giant cells. However, other features of Hodgkin's disease were not found, such as the presence of a background proliferation of reticular cells intermingled with chronic inflammatory cells of all types, including eosinophils and plasma cells; nor were binucleated giant cells in evidence.

A characteristic feature of the large atypical cells encountered in our patients was a crenated nuclear outline due to a folded or indented nuclear membrane shared with so-called mycosis fungoides cells (Brownlee and Murad, 1970; Flaxman *et al.*, 1971). However, it is unusual for mycosis fungoides cells to appear as large as the atypical cells described here; in addition, the indentation of mycosis fungoides cell nucleus can be detected with ease only in ultra-thin sections.

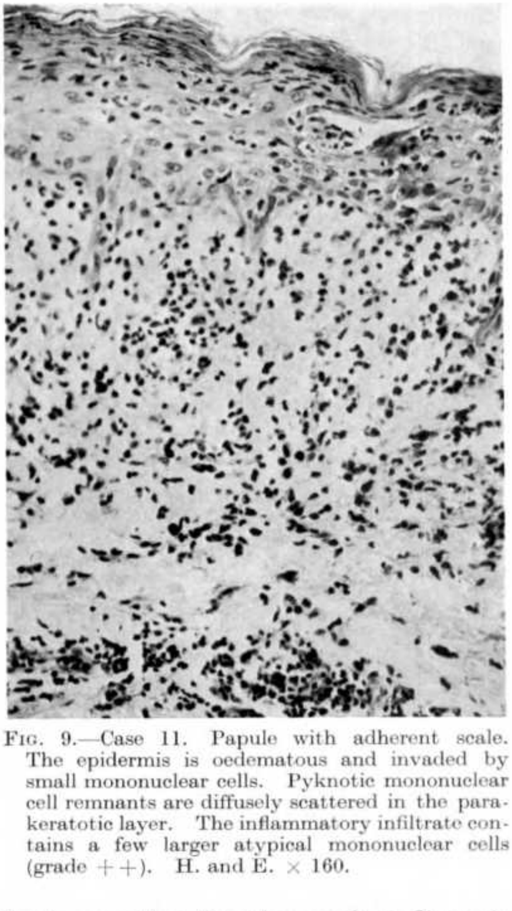

FIG. 9.—Case 11. Papule with adherent scale. The epidermis is oedematous and invaded by small mononuclear cells. Pyknotic mononuclear cell remnants are diffusely scattered in the parakeratotic layer. The inflammatory infiltrate contains a few larger atypical mononuclear cells (grade ++). H. and E. × 160.

In some of our sections individual microscopic fields closely resembled those of mycosis fungoides because of the striking permeation of the lower half of the epidermis by abnormal mononuclear cells, but the overall focal papular arrangement of the infiltrate and the evidence of inflammation and oedema with disproportionate epidermal damage were distinguishing features from mycosis fungoides.

DISCUSSION

Although the large atypical cells found in "lymphomatoid" pityriasis lichenoides undoubtedly appear to have the cytological features of malignancy, it is apparent that the course and behaviour of the disease is essentially benign. Atypical cells have also been noted in the inflammatory infiltrates that accompany benign reactive disorders such as disseminated rosacea (Marks and Wilson Jones, 1969), actinic reticuloid (Ive *et al.*, 1969) and certain types of insect bite reaction (Winer and Strakosch, 1941; Allen, 1948).

However it is only in "lymphomatoid" pityriasis lichenoides that these atypical cells become so consistently prominent in the infiltrate and thus closely simulate a malignant lymphoma. In our series the numbers of atypical cells present often varied greatly in different cases and also in separate biopsies in the same individual. Atypical cells tended to be most numerous in the early or necrotic larger papules, but conversely they were sometimes inconspicuous or even absent in the smaller papules with an adherent scale. In Case 10 the number of atypical cells in the early lesions was found to be much less after an interval of 6 years, even although the papules have continued to erupt and subside.

This study has revealed that our cases may have a protracted clinical course, with fresh papules continuing to erupt and subside for many years. In the 13 previously reported cases (see Table III) the eruption continued for longer than 5 years in 7. Despite this, the general health has been unaffected in the majority. In 2 of the published cases (see Table III) the eruption was preceded by serious systemic illness (pulmonary tuberculosis, amoebic colitis) but this was not observed in any of our 15 patients. In over half of our cases the early papules evolved into lesions which were clinically indistinguishable from those found in pityriasis lichenoides. The predominant lesions were small haemorrhagic papules that underwent necrosis before resolving to leave superficial scars, and in at least 5 cases small lichenoid papules with an adherent mica-like scale were prominent. Verralo and Haserick (1966) considered that the lesions in their 2 cases were typical of pityriasis lichenoides acuta (Mucha-Habermann disease), and this has also been commented upon by Kasumbeili (1960); Piñol Aguadé *et al.* (1967); Couperus (1967); Borrie (1969); Kawade *et al.* (1969); Muller and Schulze (1971). Larger ulcerated papules were found in 8 of our patients, and this type of lesion has also been observed in a few patients with pityriasis lichenoides in whom no atypical cells could be detected (Degos *et al.*, 1966; Burke *et al.*, 1969; Marks *et al.*, in preparation).

This study revealed also that biopsies from our cases shared many of the histological features of pityriasis lichenoides, in particular the marked oedema of the upper dermis and epidermis, together with invasion of the epidermis by mononuclear cells associated with incipient necrosis (McCarthy, 1942; Piérard and Van Steenbergen, 1957; Civatte, 1959; Szymanski, 1959; Montgomery, 1967). In 6 of our biopsies of the smaller type of lesion, in which there were few or no atypical cells, the histology was otherwise typical for pityriasis lichenoides.

The small blood vessels in the upper dermis were markedly dilated and congested with frequent haemorrhage. Swelling of the endothelial cells was a consistent feature, and deposits of fibrinoid material around vessel walls were noted in the more severe type of lesion. However, in only 1 case did we observe destruction of small vessels, which Szymanski (1959) has stressed to be an important feature of pityriasis lichenoides et varioliformis acuta. Krüger and Weise (1959) have noted leukocytoclastic changes in pityriasis lichenoides, but this was not observed in any of our sections. We suggest, therefore, that lymphomatoid papulosis is essentially a variant of pityriasis lichenoides, in which the proportion of atypical cells in the inflammatory infiltrate simulates a malignant lymphoma.

We are not sure as to the exact status of the single fatal case. For 16 years this patient's eruption, both clinically and histologically, closely resembled the findings in our other cases before larger and more severe lesions began to appear. One other fatal case showing similar features has been described from Japan (Kawada *et al.*, 1969) in which 7 years after the onset of a continuously cropping eruption of papulo-necrotic lesions, the patient began to develop larger tumours and shortly afterwards died from widespread metastatic involvement. It is clear, therefore, that prolonged follow up is necessary when the eruption continues for several years, and particularly if larger atypical lesions begin to appear.

Verrallo and Fand (1969) measured the nuclear DNA content of the atypical cells in 2 patients with lymphomatoid papulosis by Feulgen cytophotometry.

TABLE III

Authors	Sex	Age of onset	Duration (years)	Morphology of lesions	Distribution	Clinical course
Kasumbeili (1960)	M	27	11*	Haemorrhagic papules, some with adherent scale	Trunk, limbs	Alive and well
Dupont (1965)	1. M	11	31*	Erythematous scaly plaques (1–6 cm); papules (1–2 cm) becoming necrotic and leaving scars	Trunk, limbs	Alive and well 31 years after onset
	2. F	25	10*	Haemorrhagic papules; no scars. Infiltrated plaque (1·5 cm)	Trunk, limbs, face	Alive and well
Kopf and Andrade (1966)	F	45	10	Erythematous plaques. Papules, which regressed	Trunk, limbs	Alive and well
Verallo and Haserick (1966)	1. F	20	7 months	Haemorrhagic papules (3 mm to 2·5 cm), some with adherent scale	Trunk, limbs, scalp	Alive and well
	2. F	25	4 months	Haemorrhagic necrotic papules, leaving varioliform scars	Trunk, limbs	Alive and well (eruption preceded by acute amoebic colitis)
Piñol Aguadé *et al.* (1967)	M	45	10 months*	Haemorrhagic necrotic papules (2 cm), leaving scars. Smaller papules (0·5 cm), with adherent scale	Trunk, limbs	Alive and well
Couperus (1967)	M	18	1*	Haemorrhagic papules (0·6 cm), with larger ulcero-necrotic lesions (2–3 cm), leaving scars	Trunk, upper eyelids	Alive and well

Berardi *et al.* (1968)	F	47	4 months	Haemorrhagic scaly papules, some becoming necrotic and leaving scars	Trunk, limbs	Alive and well
Borrie (1969)	F	19	15*	Haemorrhagic papules (0·5–1 cm), becoming necrotic and leaving scars	Trunk, limbs	Alive and well 15 years after onset. PH: asthma; hay fever
Kawada *et al.* (1969)	1. M	22	8	Haemorrhagic papules (0·5–1 cm); ulcerated tumours (5 cm)	Trunk, limbs, neck	Eruption preceded by pulmonary tuberculosis and treated with streptomycin. Died 8 years after onset. At autopsy diffuse sarcomatous involvement in tonsils, liver, many lymph nodes and bone marrow
	2. M	42	11*	Haemorrhagic papules (0·5 cm), some becoming necrotic	Trunk, limbs	Alive and well 11 years after onset
Muller and Schulze (1971)	F	68	3*	Haemorrhagic papules (0·5 cm–2 cm), tending to become necrotic and leave scars	Trunk, limbs	Alive and well 3 years after onset. PH: rheumatoid arthritis and myxoedema

* Still getting new lesions.

They found that the DNA content of the larger atypical cells tended to be hypertetraploid, a feature which has been considered in other situations to be indicative of malignancy (Kint, 1963). Verrallo and Fand could detect no cytophometric evidence of cell renewal in the large atypical cells and suggested that they were destroyed, possibly by an immunological mechanism.

Our preliminary histochemical findings indicate that the large atypical cells are rich in hydrolytic enzymes. Brounstein *et al.* (1962) have shown that, whereas histiocytes show abundant hydrolytic enzyme activity, both the blast cells in leukaemia and Hodgkin's giant cells had no or weak reactions. These findings indicate that the large atypical cells found in our cases may be derived from histiocytes, and are probably distinct from, both blast cells and Hodgkin's giant cells. The factors which stimulate the formation of these large atypical cells are completely unknown.

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ULTRASTRUCTURAL ASPECTS OF DERMATITIS HERPETIFORMIS (DUHRING'S DISEASE)

F. GUERRA RODRIGO

Summary.—Biopsies of clinically normal skin and erythematous and recent vesicular lesions of 3 patients with dermatitis herpetiformis (DH) were studied by electron microscopy.

The early events at the dermo-epidermal junctional zone were the presence of glycogen and lipid particles in the basal cells, rarefaction of half-desmosomes, and small duplications of the basal lamina.

In some areas of the dermo-epidermal junction of the erythematous skin separation between epithelial basal cells and basal lamina was observed. In the vesicles fibrin, with its typical cross-banding pattern, was seen.

The early changes observed in the epithelial germinative cells were interpreted as evidence of disturbance in dermo-epidermal interaction, possibly caused by immunoglobulin deposition at this level. The abnormal synthesis of half-desmosomes by the basal cells could be the cause of supra-laminar disjunction.

DERMATITIS herpetiformis (DH) is a relapsing blistering dermatosis of unknown etiology, with a characteristic clinical picture. Histopathology of the skin lesions provides the main procedure for confirmation of the diagnosis. However, the histogenic interpretation of the cutaneous changes is not clear, particularly at the ultrastructural level. In the present work biopsies performed in 3 patients with DH were examined by electron microscopy to investigate the pathomechanism of the skin lesions.

MATERIAL AND METHODS

Three patients with DH were studied. In each the clinical picture was characteristic, and the diagnosis was confirmed by histological examination. Indirect immunofluorescent tests for detection of serum circulating anti-epithelial antibodies (pemphigus and pemphigoid antibodies) were negative.

The biopsies were performed prior to the beginning of therapy. Three different areas were selected for the biopsies: normal skin near the typical lesions (2 to 4 cm from the lesions); erythematous and infiltrated skin without vesicles; recent vesicles (less than 24 hours duration).

The biopsies were taken under local procaine anaesthesia. They were fixed in 3% glutaraldehyde, followed by 2% osmium tetroxide (Millonig's buffer, pH 7·3). After embedding (Epon 812), thin sections were cut (Ultratome LKB) and stained (uranyl acetate-lead citrate). They were observed in an Elmiskop 1A at 80 kW.

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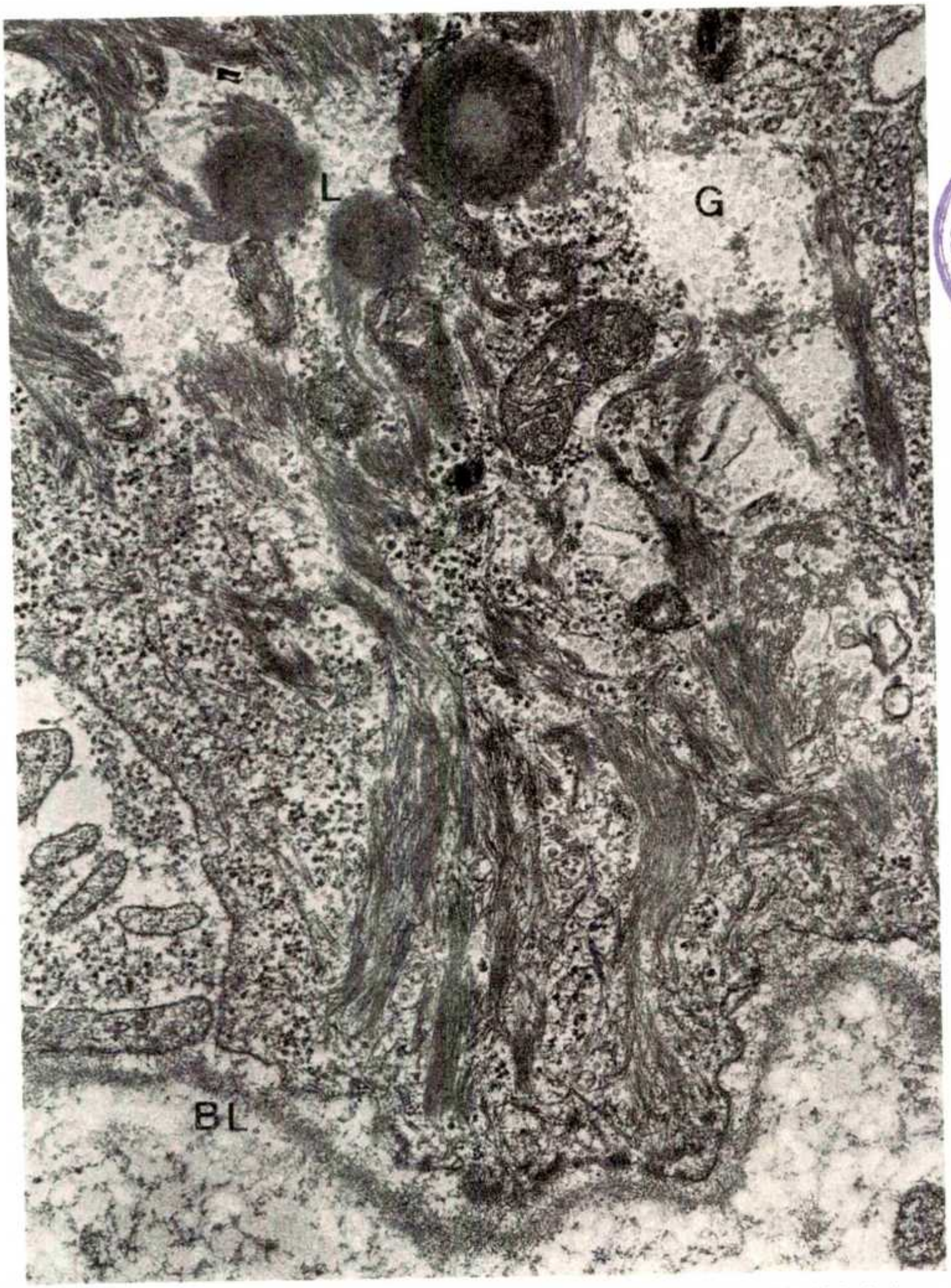

FIG. 1.—Erythematous skin: dermo-epidermal junction. Glycogen (G) and lipid inclusions (L) are present in the cytoplasm of a basal cell. BL = basal lamina. (×32,000.)

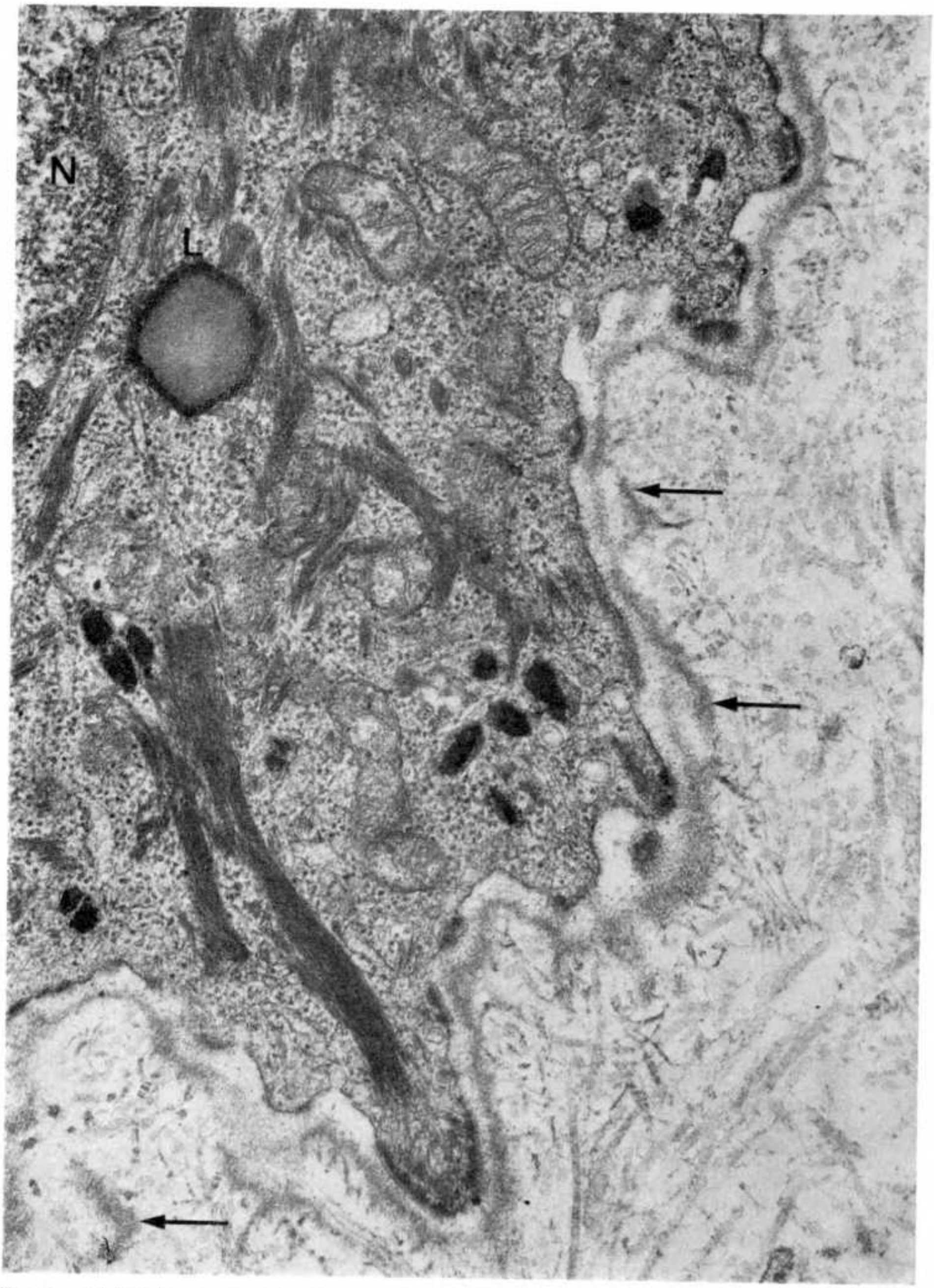

Fig. 2.—Clinically normal skin: dermo-epidermal junction. In the cytoplasm of the basal cell a lipid inclusion (L). Note the small duplications of the basal lamina (arrows). N = nucleus. (× 32,000.)

RESULTS

In the study of the biopsies, particular attention was focused on the dermo-epidermal junctional zone (basal cells, basal lamina and papillary dermis), where pathological events were presumed to be more significant.

The ultrastructural changes observed in the 3 patients were essentially the same. In the clinically normal skin the abnormalities were slight and inconstant. They were much more evident in the erythematous skin, and they were intense and mainly degenerative in the blistering lesions.

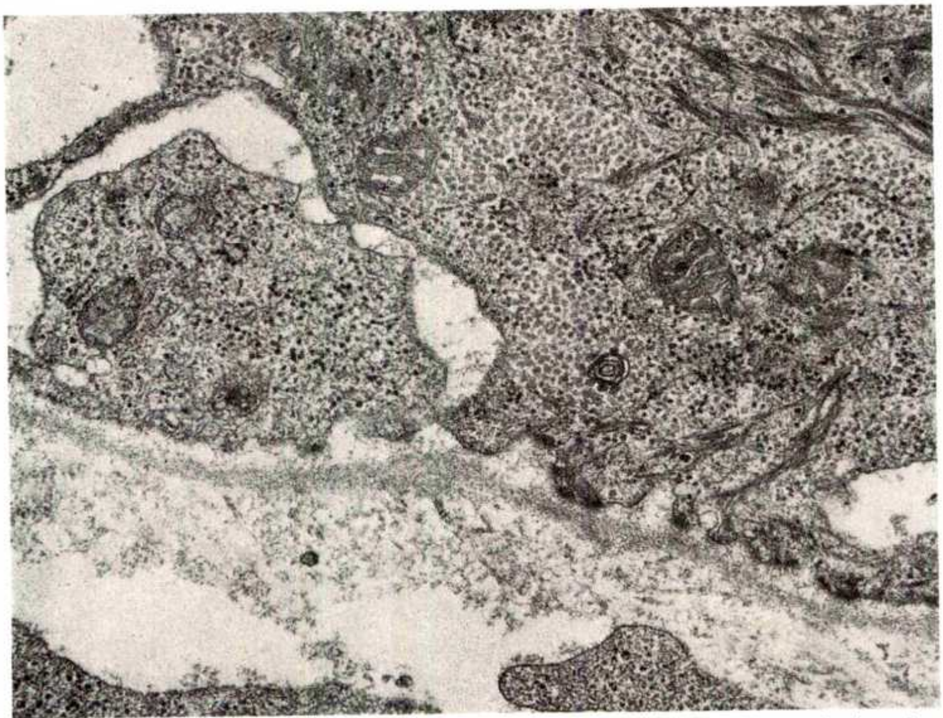

FIG. 3.—Erythematous skin: dermo-epidermal junction. In this area the rarefaction of half-desmosomes is evident. (× 32,000.)

Basal cells.—In the normal and erythematous skin the general cyto-architecture was well preserved, and the morphology of the cell organelles was normal. Lipid and glycogen inclusions were observed in the cytoplasm of these cells, more frequently in the erythematous skin biopsies (Fig. 1). The inclusions were identified by their morphological characteristics. The lipid droplets were round and 0·15 to 0·6 μm in diameter. Sometimes a dense line around the periphery of these droplets was seen (Fig. 2). They were similar to the " irregular dense bodies " described by Nix (1967) in the basal layer of u.v.-irradiated skin. Glycogen particles 15 to 30 nm in diameter, with an irregular outline (β-type), were present, sometimes in large amounts.

In the vesicles considerable degenerative changes were always observed. The basal cells were flattened, sometimes disrupted, and the cytoplasmic organelles were vacuolated and disorganized. The tonofilaments were well preserved and

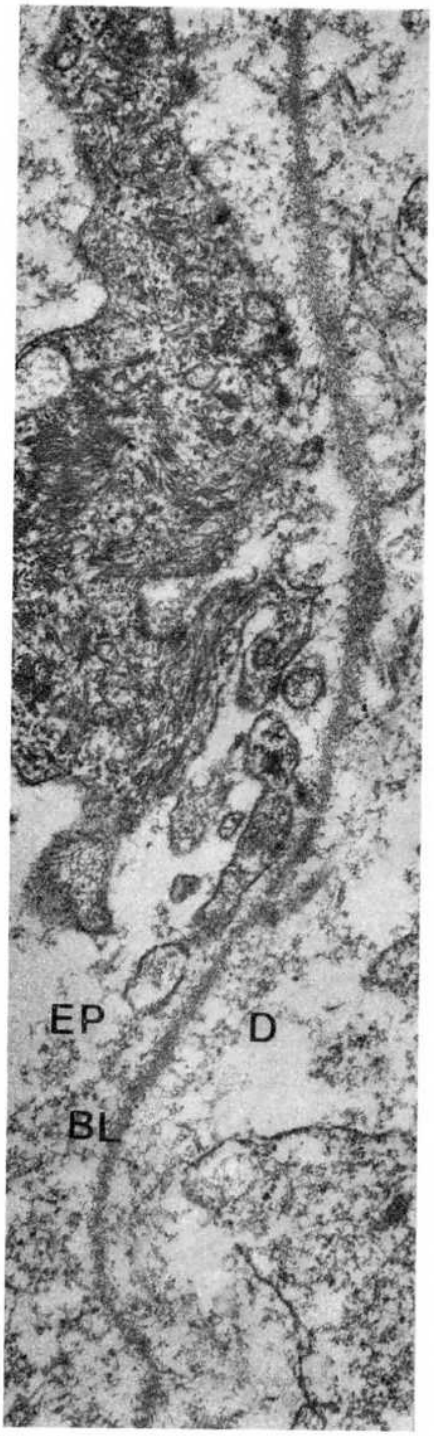

FIG. 4.—Erythematous skin: dermo-epidermal junction. Basal lamina (BL) detached from the basal cells. EP = epidermis. D = dermis. (×38,400.)

they could be seen forming compact and irregular bundles among cell fragments.

Basal lamina.—Three main types of abnormality could be observed:

(1) Duplication of short segments of the lamina in the normal and the erythematous skin;

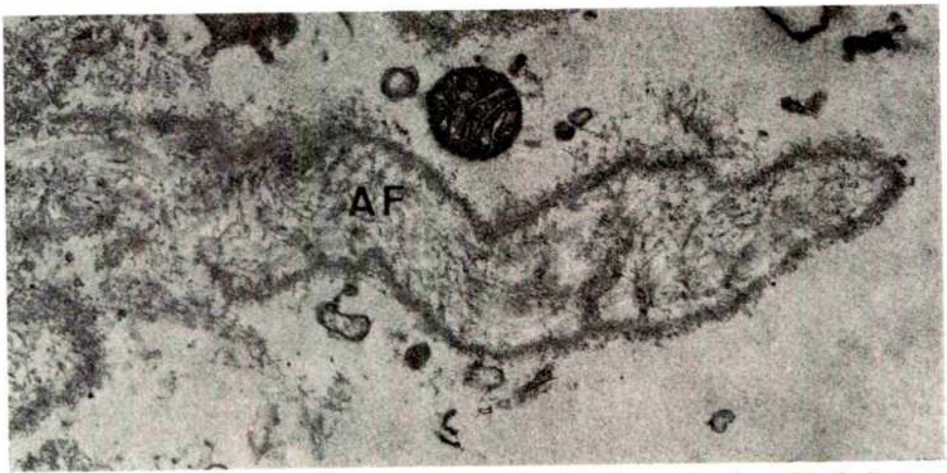

FIG. 5.—A fragment of basal lamina intermingled with cellular fragments observed in the lumen of a bullous lesion. The connections of the lamina with the basal cells are lost, but the connections with the anchoring fibrils (AF) are maintained. (× 38,400.)

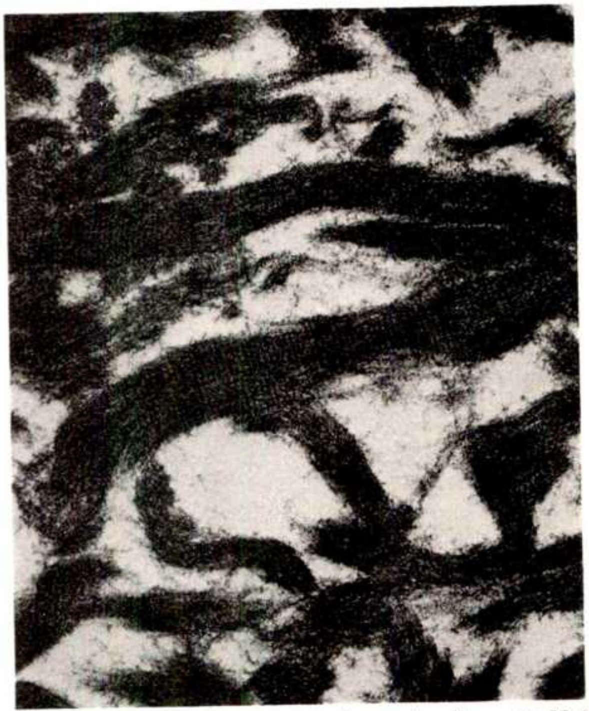

FIG. 6.—Fibrin strands with the typical cross banding. (× 38,400.)

(2) Disappearance of the lamina in some areas, forming gaps where inflammatory cells stretched out from the dermis into the epidermis;

(3) Loss of adherence between the lamina and the basal cells, in which the half-desmosomes were rarefied or absent (Fig. 3, 4).

Papillary dermis.—The characteristic aspects of the erythematous skin and the blistering lesions were oedema and inflammatory cells, with predominance of eosinophils.

In the areas where dermo-epidermal separation was complete some fragments of the basal lamina, intermingled with fibrin-like material, cellular fragments and inflammatory cells, could be observed. In the dermal face of these fragments anchoring fibrils were present (Fig. 5). Clusters of fibrin-like, osmiophilic material were abundant in the dermo-epidermal hollow, showing, in suitable orientation, a cross-banding with a periodicity of 22 to 26 nm (Fig. 6).

DISCUSSION

In human skin the attachment of the epithelium to the underlying dermis is firm. The binding structures are the epithelial basal cells and the papillary dermis, interconnected through the basal lamina, where the half-desmosomes and the anchoring fibrils are implanted. In this dermo-epidermal junctional zone, experimental disjunction is difficult (Küstala and Mustakallio, 1967) in striking contrast to the easy separation which can be observed in some blistering dermatoses, as in DH. The mechanism of dermo-epidermal separation in this disease is unknown, and the dynamic sequence of the pathological events is not clear.

Electron microscope studies of the cutaneous lesions of DH have been performed in the last years (Bellone and Caputo, 1966; Piérard and Kint, 1968; Fry and Johnson, 1969; Mustakallio *et al.*, 1970; Jakubowicz *et al.*, 1970, 1971). The importance of the destruction of the basal lamina has been stressed recently by Jakubowicz *et al.* (1970, 1971), who considered this finding the characteristic event of DH. Bellone and Caputo (1966) had previously suggested that the changes in the papillary dermis and the disappearance of the basal lamina were the *primum movens* of the dermo-epidermal separation. These authors, studying recent bullous lesions, described the disappearance of the basal lamina in the periphery of the bulla, but they could frequently find it in the floor of the cavity. In order to explain this fact they speculated that it might be the result of the dermal regeneration of this structure. This hypothesis has, however, little support from experimental data, which are more compatible with the concept that the basal lamina is synthesized by the epithelial cells (Pierce and Nakane, 1969).

It is known that the dermis is necessary for epidermal control (Briggaman and Wheeler, 1968), and when dermo-epidermal connection is lost, degenerative and inflammatory phenomena progress quickly. In vesicular lesions, even recent ones, degenerative changes are already very intense. For this reason, the specificity and the chronology of the alterations are difficult to establish. Thus, in the present work, careful attention was focused on the clinically normal skin near the typical lesions and on areas of the erythematous skin, where dermo-epidermal separation was not yet apparent or was just beginning.

The early characteristic event was the accumulation of glycogen particles and lipid droplets in the cytoplasm of the basal cells, as well as the presence of small areas of duplication of the basal lamina. Rarefaction of half-desmosomes was also evident in some areas. The finding of these abnormalities in basal cells of the normal skin, and more clearly in the erythematous skin, suggests that the influence of the dermis on the epidermis is disturbed, leading to a metabolic disturbance of the germinative cells. We can speculate that this change may be the cause of abnormal synthesis or maintenance of the half-desmosomes. The supra-laminar separation can be explained by this mechanism. The presence of the basal lamina in the floor of the bulla, as described by Bellone and Caputo (1966), probably represents another instance of the early supra-laminar disjunction in this disease.

Inflammatory cells are probably attracted by chemotatic stimuli arising in the damaged epidermis. It is known that antigen–antibody complexes have this effect upon the eosinophils (Litt, 1964). The eosinophil predominance in the infiltrate suggests that an antigen–antibody reaction may be one of the stimuli. The recent demonstration of deposition of immunoglobulin A in the dermo-epidermal junction of normal skin in DH (Van der Meer, 1969; Jablonska, 1970), supports this hypothesis and leads to the assumption that immunological phenomena at the junction level may be the primary event in the pathomechanism of the lesions.

Deposition of fibrin-like material in the skin lesions of DH is another important characteristic of this disease (Mustakallio *et al.*, 1970). In the present work it could be found only when dermo-epidermal disjunction was already complete, and was associated with important cellular degenerative changes of the epidermis. In some strands of this material a cross-banding pattern typical of fibrin (Hall, 1963) was observed (Fig. 6). This cross-banding was not found by Jakubowicz *et al.* (1971), who argued that the absence of bands is probably due to change in the fibrin occurring in DH.

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VARIATIONS IN SKIN SURFACE LIPID COMPOSITION AND SEBUM EXCRETION RATE WITH DIFFERENT SAMPLING TECHNIQUES II

J. A. COTTERILL, W. J. CUNLIFFE AND B. WILLIAMSON

Summary.—The technique of Sellotape stripping was used to investigate the role of the stratum corneum in modifying sebum excretion rate and skin surface lipid composition.

All experiments were performed on the forehead. The stratum corneum influences the rate of sebum excretion as Sellotape stripping produced a significant increase in sebum excretion rate, but this effect was abolished by systemic antibiotics.

Skin surface lipid composition, when sampled by absorbent papers, was not influenced by Sellotape stripping, but when sampled by an ether-soaked sponge after Sellotape stripping, had a lessened epidermal lipid contribution. Thus the stratum corneum modifies skin surface lipid composition when sampled by an organic solvent.

Absorbent papers may be preferable to other sampling techniques on the forehead in the investigation of the role of sebaceous biochemical factors in the pathogenesis of acne vulgaris.

THE skin surface lipid composition of the forehead varies considerably with the method of collection (Cunliffe *et al.*, 1971). When sampled by an organic solvent the surface lipid has a composition which reflects a greater epidermal contribution than does surface lipid collected by absorbent papers which sample lipid with a greater contribution from the sebaceous glands (Cunliffe *et al.*, 1971). In the present study we have investigated the role of the outermost part of the epidermis in modifying skin surface lipid composition.

MATERIAL AND METHODS

We investigated 41 patients, 23 females and 18 males, all of whom had varying degrees of acne vulgaris. Their ages ranged from 14 to 31 years. Eighteen patients had been taking either tetracycline 250 mg, trimethoprim-sulphamethoxazole (80 mg trimethoprim and 400 mg sulphamethoxazole) or Clindamycin 150 mg daily for at least 1 month prior to the investigation, whereas none of the other subjects was receiving any topical or systemic therapy. The patients washed their hair on the evening preceding the collection, but did not wash the face on the morning of the test.

In 21 patients, including 8 who were receiving antibiotics, absorbent papers were applied to both sides of the forehead until a follicular pattern was obtained. At this stage of the experiment one side of the forehead had the outermost part of its stratum corneum removed by using 1·5 in. wide transparent adhesive tape (Sellotape). The tape was pressed firmly on to the side and then removed, taking with it keratinocytes and

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bacteria. The stripping procedure was repeated 25 times, and the surface lipid was then collected from both sides of the forehead for 3 hours, using absorbent papers.

In another group of 20 patients, 10 taking antibiotics, a follicular pattern was again obtained on the forehead, and one side was likewise stripped 25 times with Sellotape. On the unstripped side sebum was collected by absorbent papers for 3 hours, but during this time period the Sellotape-stripped side was left exposed to the atmosphere. At the end of 3 hours the surface lipid was collected from the unstripped side, using 10 firm strokes with an Analar ether-soaked polyurethane sponge.

TABLE I.—*Differences in Skin Surface Lipid Composition and Sebum Excretion Rate between the Unstripped and Stripped Sides (+ = Stripped, > Unstripped; — = Unstripped, > Stripped). Skin Surface Lipid Was Collected on Each Side by Absorbent Papers. No Subject Was Taking Antibiotics*

Subject	Sebum excretion rate ($\mu g/cm^2/min$)	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	0·00	−2·0	−3·2	−9·0	+14·1	+0·2
2	−0·12	+1·6	−3·1	−5·3	+6·9	−0·1
3	−0·07	+3·9	+0·4	−10·2	+6·2	−0·3
4	−0·38	+0·1	−1·4	−0·8	+2·1	0·0
5	+0·02	+0·4	+2·5	+4·3	−6·6	−0·5
6	−0·34	+0·2	−0·2	−5·0	−4·7	−0·1
7	−0·14	+1·6	−0·8	−1·5	+0·6	0·0
8	+0·08	−1·5	−3·2	+10·2	−5·1	0·0
9	+0·06	+2·8	−0·7	+5·3	−7·3	−0·1
10	−0·37	−3·4	−5·8	+0·5	+8·2	0·0
11	+0·01	0·0	+1·3	−4·4	+3·4	0·0
12	−0·22	−3·1	+0·5	−19·3	+21·8	0·0
13	−0·14	+0·1	+4·0	−6·9	+2·9	0·0
A.M.	−0·13	+0·05	−0·71	−3·24	+3·99	−0·07
S.E.M.	0·05	0·08	0·74	2·11	2·25	0·04
t	2·85	0·08	−0·96	−1·53	1·77	−1·75
2p	0·02	0·90	0·30	0·1	0·1	0·1

TABLE II.—*Differences in Skin Surface Lipid Composition and Sebum Excretion Rate between the Unstripped and Stripped Side of the Forehead in 8 Subjects Receiving Antibiotics (+ = Stripped, > Unstripped; — = Unstripped, > Stripped). Skin Surface Lipid Was Collected on Each Side by Absorbent Papers*

Subject	Sebum excretion rate ($\mu g/cm^2/min$)	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	+0·32	−0·2	+1·9	−2·0	+0·2	+1·0
2	+0·20	+0·5	−2·4	−3·6	+6·8	−0·5
3	+0·18	−1·2	−0·6	+0·9	+0·1	−0·2
4	−0·59	−3·7	+3·6	+1·8	−1·5	−0·1
5	0·58	+1·2	+2·3	−3·1	−0·4	+0·1
6	−0·13	+2·2	+12·2	−18·5	+3·8	+0·2
7	−0·21	+2·8	+17·8	−24·0	+3·6	−0·1
8	+0·09	+1·8	−1·8	+1·7	−1·5	−0·1
A.M.	−0·09	+0·42	4·12	−5·85	+1·39	0·03
S.E.M.	0·12	0·75	2·54	1·68	1·06	0·15
t	−0·72	0·56	1·62	−1·68	1·31	0·20
2p	0·4	0·5	0·1	0·1	0·2	0·8

The samples from the polyurethane sponge and paper were extracted, using 3 aliquots of 25 ml Analar ether. All samples were then immediately evaporated, using a rotary evaporator (Büchi) (Cotterill *et al.*, 1971a). The samples were flushed with nitrogen after weighing and then stored at − 20°C until the following day, when semiquantitative biochemical analysis was performed (Cotterill *et al.*, 1971a).

RESULTS

Table I shows the difference in sebum excretion rate in patients in whom the effect of Sellotape stripping on samples collected by papers alone was investigated.

TABLE III.—*Differences in Skin Surface Lipid Composition when Data Analysed After Collection by an Ether-soaked Sponge from the Sellotape-stripped Side of the Forehead were Subtracted from Data Obtained After Collection by Absorbent Papers from the Unstripped Side in* 10 *Subjects not Receiving Antibiotics* (+ = *Stripped*, < *Unstripped*; − = *Unstripped*, < *Stripped*)

Subject	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	+5·1	+3·1	+9·2	−17·3	−0·1
2	+1·8	−0·4	+3·4	+2·1	−0·1
3	+2·7	+2·9	+11·8	−17·1	−0·1
4	+0·7	−1·4	+12·7	−12·0	0·0
5	−3·3	−10·0	+8·5	+5·0	−0·2
6	+0·7	−1·4	+10·6	−8·7	−1·1
7	+3·4	+3·1	+11·3	−16·0	−1·9
8	+1·9	+4·6	−4·8	−1·5	−0·3
9	−4·2	+5·6	+5·1	−6·2	−0·4
10	+7·7	−8·4	+11·5	−12·1	+1·2
A.M.	+1·65	−0·23	+7·25	−8·38	−0·30
S.E.M.	1·12	1·68	2·01	2·54	0·25
t	1·47	−0·14	3·60	−3·30	−1·19
2p	0·1	0·8	0·01	0·01	0·2

TABLE IV.—*Differences in Skin Surface Lipid Composition when Data Analysed After Collection by an Ether-soaked Sponge from the Sellotape-stripped Side of the Forehead Subtracted from Data Obtained After Collection by Absorbent Papers from the Unstripped Side in* 10 *Subjects Taking Antibiotics* (+ = *Stripped*, < *Unstripped*; − = *Unstripped*, < *Stripped*).

Subject	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acid (%)	Cholesterol (%)
1	+1·1	+0·3	−0·2	−0·6	−0·7
2	+2·8	+1·4	+7·1	−9·7	−1·7
3	+1·6	+9·0	+14·5	−24·7	−0·4
4	−0·2	+1·8	+6·4	−7·6	−0·5
5	−1·1	−10·6	+12·0	−0·1	−0·1
6	+1·4	−4·9	+7·3	−2·8	−1·0
7	−1·2	−0·7	+10·5	−5·6	0·0
8	+3·2	−2·6	+13·5	−16·9	0·8
9	+0·7	+2·3	+6·6	−3·8	−0·5
10	−1·2	−3·7	+2·4	−3·5	−0·1
A.M.	+0·71	−0·77	+8·01	−7·53	−0·4
S.E.M.	0·51	1·64	1·49	2·46	0·21
t	1·39	−0·47	5·38	−3·06	−1·95
2p	0·1	0·6	0·001	0·02	0·1

When the subjects were not receiving antibiotics, the sebum excretion rate was significantly greater on the Sellotape-stripped side ($P < 0{\cdot}02$), but this difference was abolished in the antibiotic-treated subjects (Table II). In these subjects Sellotape stripping produced no significant changes in the percentages of squalene, wax ester, triglycerides, free fatty acids and cholesterol in either the antibiotic or non-antibiotic groups (Tables I, II).

Certain differences were found in the second group of 20 subjects in whom the surface lipid was collected by absorbent papers and compared to the lipid collected by sponging from the side which had been Sellotape-stripped (Tables III, IV). There were significantly greater percentages of triglyceride and significantly less free fatty acids in the paper samples. There were no significant differences in squalene, wax ester and cholesterol between the 2 sides, in either the antibiotic or non-antibiotic groups, and the probabilities are shown in Tables III, IV.

DISCUSSION

We have previously shown, using absorbent papers that there are no significant differences in sebum excretion rate (Cunliffe and Shuster, 1969) or skin surface lipid composition (Cotterill *et al.*, 1971a) between the right and left side of the forehead.

We used Sellotape stripping to assess the effect of the stratum corneum on sebum excretion rate and skin surface lipid composition. We accept that Sellotape stripping is perhaps not the ideal way of removing stratum corneum, but we were anxious not to use cyanoacrylate adhesive (Marks and Dawber, 1971) near the eyes. Furthermore, we do not know the effect of temperature on skin surface lipid composition; an increase of temperature is employed by Turek (1971) in his technique for the removal of stratum corneum. We have shown that Sellotape stripping produces an increase in sebum excretion rate, an effect which is abolished by antibiotics. In the acne patients not receiving antibiotics it is possible that Sellotape stripping will relieve some of the obstruction to sebum outflow by removal of the hyperkeratotic plugs which are characteristic of this condition. There is evidence in rabbits that sebaceous lipids and in particular free fatty acids may produce follicular hyperkeratosis (Kligman and Goldman, 1968), and those broad spectrum antibiotics which are effective in acne are known to reduce the free fatty acid composition of skin surface lipid (Beveridge and Powell, 1969; Cotterill *et al.*, 1971b). Thus long term treatment with antibiotics may be associated with less marked follicular plugging so that Sellotape stripping would, as we have found, have no effect on sebum excretion rate. It follows that long-term effective antibiotic treatment should perhaps increase sebum excretion rate, but there is no evidence that this in fact happens. However, our observations are in keeping with the idea that the cutaneous bacterial flora in acne subjects may lead directly or indirectly to follicular obstruction. An alternative explanation for the increased sebum excretion rate in patients not taking antibiotics could be an effect of the stripping on sebum production. Repeated Sellotape stripping is known to increase epidermal mitosis (Pinkus, 1952), but the slow turnover of sebaceous cells (Epstein and Epstein, 1966) and the failure to find any difference in the antibiotic treated group makes this explanation highly unlikely.

The stripping procedure had no significant effect on skin surface lipid composition sampled by absorbent papers, and thus the composition of surface lipid sampled by this method is not influenced by the presence of the outermost layer of the skin. This finding lends support to our contention that absorbent papers sample lipid more closely approaching pure sebum in composition than do other sampling techniques. We realize that there may be selective loss of lipids for subsequent analysis when samples are taken by absorbent papers, but precise data on this possibility is not available, and this problem is under active investigation.

In the present studies sponging after Sellotape stripping sampled significantly more free fatty acids and significantly less triglyceride than the absorbent papers. However, there were no significant differences in squalene, wax ester and cholesterol. This is in striking contrast to our previous work on sponged unstripped skin (Cunliffe *et al.*, 1971), where we have shown that samples of skin surface lipid obtained by the sponge technique as compared to the paper technique contained not only significantly less triglyceride and more free fatty acid but also significantly less wax ester and squalene and significantly more cholesterol. We interpreted these findings to mean that the sebum collected by absorbent papers approximates more to the composition of pure sebum than that collected by sponging, which has a greater epidermal contribution. It is clear that samples obtained by sponging after Sellotape stripping eliminate the differences we have previously found in wax ester, squalene and cholesterol and sample skin surface lipid, with a lesser epidermal contribution than that from unstripped skin.

We conclude that the stratum corneum influences the rate of sebum excretion in untreated acne subjects, probably by modifying resistance to sebum outflow, and that absorbent papers may be preferable to other sampling techniques on the forehead in the investigation of the role of sebaceous biochemical factors in the pathogenesis of acne vulgaris.

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PHYSICO-CHEMICAL FACTORS INFLUENCING EPIDERMAL DAMAGE BY SURFACE ACTIVE AGENTS

A. B. G. LANSDOWN AND P. GRASSO

Summary.—The responses of mouse skin to single (10%) and repeated (1% or 2·5%) applications of sodium lauryl sulphate (SLS), cetrimide and Tween-80 were examined visually and histologically after various periods of exposure. Whereas no damage was caused by Tween-80 when given as single applications or daily for 9 days, SLS and cetrimide both produced damage. After single applications the changes consisted of epidermal cell degeneration, focal necrosis and dermal inflammatory cell infiltration. After repeated treatment acanthosis and hyperkeratosis were identified, and nuclear debris was present in the keratin layer. Cetrimide-treated skins were more severely damaged than those treated with SLS. In general, histological changes preceded visual changes by 1 to 2 days.

Irritancy due to these surfactants was related to their physico-chemical properties. Since Tween-80 at the concentrations used was highly surface active and lipophilic, these properties were not considered of importance in explaining the damage due to SLS or centrimide. Both of these agents react well with protein such as keratin whereas Tween-80 does not. Thus it would seem that this property is of primary importance in the production of damage.

Ionic and non-ionic surface active agents are included in a wide range of cosmetic, household and industrial preparations, in view of their emulsifying and detergent properties. A number of surfactants are known to possess irritant properties when applied to the skin of animals or man and, for this reason, their effects on the skin have been extensively studied under both *in vitro* and *in vivo* conditions (Blank, 1939; Emery and Edwards, 1940; Van Scott and Lyon, 1953; Mezei *et al.*, 1966; Malaszkiewicz and Gloxhuber, 1970). As a result of this work it was shown that several biochemical changes may occur when surfactants come into contact with the skin. According to Smeenk (1969) these included change of the pH, removal of surface lipids or water soluble substances, and denaturation of the proteins in the horny layer. The uncertain relationship of these effects to the occurrence of morphological changes in the skin (Stupel and Szakall, 1957) led Smeenk (1969) and Smeenk and Polano (1965) to investigate a correlation between the irritancy of a number of surfactants and their ability to denature proteins. For this work, 6 anionic, 2 non-ionic and 1 amphoteric detergents in common use in household products were selected and their irritancy examined in human volunteers, using patch tests and repeated arm immersion tests. A parallel series of biochemical studies were carried out which included the extraction of amino acids from the skin during simulated washing tests, the effect of the compounds on available sulphydryl groups in isolated callus, and the influence of

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the compounds on the permeability of isolated human epidermis. In general, no correlation appeared in these experiments between the irritancy observed and the results of the biochemical tests, but the permeation characteristics seemed to correlate well with patch test results (Smeenk, 1969).

Since Smeenk (1969) carried out his irritancy tests on humans, histological examination of the treated skins was not practicable. It is possible that the lack of correlation may possibly be due to a considerable observer error inherent in these studies.

In the present work we have investigated the relationship between the skin irritation produced by 3 surfactants and the ability of these substances to denature the epidermal protein in mouse skins. Three surfactants were selected whose physical properties and mode of interaction with protein are well known. They were applied by the " open " patch test technique since the " occlusive " patch as advocated by Draize (1965) is known to increase the state of hydration of the skin and the permeability of the stratum corneum (Rostenberg, 1961). The sequential changes produced in the skin by repeated application of the surfactants were also studied, using detailed histological techniques.

EXPERIMENTAL

Materials.—The sodium lauryl sulphate (B.P.) and cetyl trimethyl ammonium bromide (Cetrimide) used in this study were obtained from British Drug Houses Ltd., Poole, Dorset, and the Tween-80 (polysorbate-80) from Honeywell Atlas Ltd., Carshalton, Surrey.

Animals and diet.—Female mice of the TF1 strain of the original Carworth Farm stock of albino mice, bred by A. Tuck (Rayleigh, Essex) were used in all sections of this work. They were randomly selected animals about 8 weeks old. At this age the hair coat was judged to be in the second resting period (telogen) from the data given by Hardy (1949) for the albino mouse, and by Chase (1954) in a general review on hair growth. To minimize the possible disturbances in epithelial growth due to changes in the diurnal rhythm referred to by Bullough and Laurence (1966) and Major (1970), the mice were maintained under a strict daily routine throughout (18 hours light, 6 hours dark). A diet consisting of standard rodent breeding pellets (Oxoid) and water was available at all times.

Determination of the local irritancy of the surfactants.—In these experiments the mice were shorn in the post-scapular back region with electric clippers at 10.0 a.m., 24 hours prior to treatment. Care was taken to avoid damaging the epidermal tissues, but, where damage occurred, animals were discarded. To study the responses of the skin to the surfactants, 0·5 ml of the agent was pipetted on to the back and the droplet spread out with a glass rod over an area of approximately 2 cm^2. For each surfactant 2 groups of 15 animals were used and treated as follows.

Group 1—single application of the surfactant given (10% w/v in distilled water).
Group 2—repeated applications of the surfactant given daily for 2, 5 or 9 days (1% or 2·5% w/v in distilled water).

Control animals were treated with an equivalent number of applications of distilled water. In both treated and control groups the mice were restrained until the applications had dried. No occlusive dressings were used.

In Group 1, 5 animals were killed by cervical dislocation and tissue samples obtained 30, 60 and 120 min after treatment. Tissue samples from the animals in Group 2 were obtained from animals killed 24 hours after the final treatment. The samples of treated skin were fixed in phosphate buffered formalin (pH 7·0) for routine histological examination.

Three representative strips from each piece were dehydrated in graded alcohols and embedded in paraffin wax in the usual way. Sections 5–7 μ thick were stained with H. and E., Weigart's haematoxylin and van Geisen's counterstain for collagen and by the P.A.S. technique (Pearse, 1968).

RESULTS

Single application studies.—In mouse skins treated with a single application of sodium lauryl sulphate or cetrimide the visual changes consisted of a slight thickening or puffiness of the treated areas and erythema which appeared 1·5 to 2 hours after treatment. With Tween-80, however, the skins were normal in appearance up to 2 hours after treatment.

TABLE I.—*Histological Changes Seen in Mouse Skins Treated with a Single Topical Application of Sodium Lauryl Sulphate, Cetrimide or Tween-80*

Treatment	Intracellular oedema	Nuclear pyknosis	Cellular degeneration and necrosis
Sodium lauryl sulphate (10%)			
30 min	+*	—	—
60 min	++	+	—
120 min	++	++	+
Control (120 min)	—	—	—
Cetrimide (10%)			
30 min	±	—	—
60 min	++	++	+
120 min	++	++	++
Control (120 min)	—	—	—
Tween-80 (10%)			
30 min	—	—	—
60 min	—	—	—
120 min	—	—	—
Control (120 min)	—	—	—

* The changes seen affected all animals in the groups.
± Only occasional cells involved; + 10–30% cells involved; ++ 40–90% cells involved.

The histological changes seen in the mouse skins treated with the 3 agents are given in Table I. With sodium lauryl sulphate or cetrimide, 30–40% of the squamous cells of the epidermis exhibited an intracellular vacuolation or oedema when examined 30 min after treatment. This oedema was identified as a " clear " zone in the perinuclear cytoplasm (Fig. 1). One hour after treatment the histological profile was similar, except that as many as 80–90% of the epidermal cells were oedematous and some contained nuclei that were more darkly stained than usual with haematoxylin, suggesting pyknosis. Despite these cellular changes in the epidermis, the stratum corneum was histologically normal.

Two hours after treatment with sodium lauryl sulphate or cetrimide the microscopic changes were more advanced than those identified after 1 hour. In the epidermis numerous foci of cellular degeneration and necrosis were evident (Fig. 2) and dermal changes consisted of oedema, leucocyte infiltration and some engorgement of the more superficial blood vessels. No histological evidence of damage to the dermal collagen was present 2 hours after a single application of these agents.

Histological changes were absent in mouse skins given a single application of 10% Tween-80 up to 2 hours prior to killing.

Repeated application studies.—The sequence of appearance of visual changes in

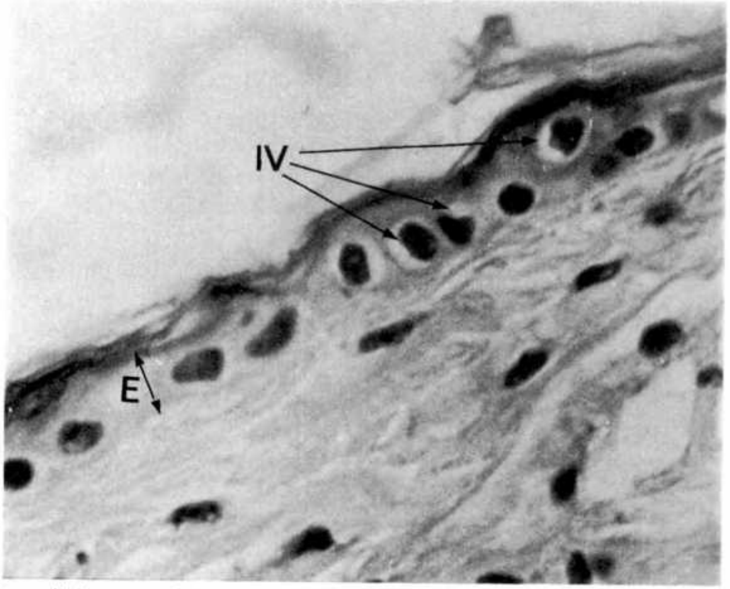

FIG. 1.—Intracellular vacuolation in mouse epidermis treated with sodium lauryl sulphate for 30 min. IV, intracellular vacuolation; E, epidermis (H. and E. × 1000).

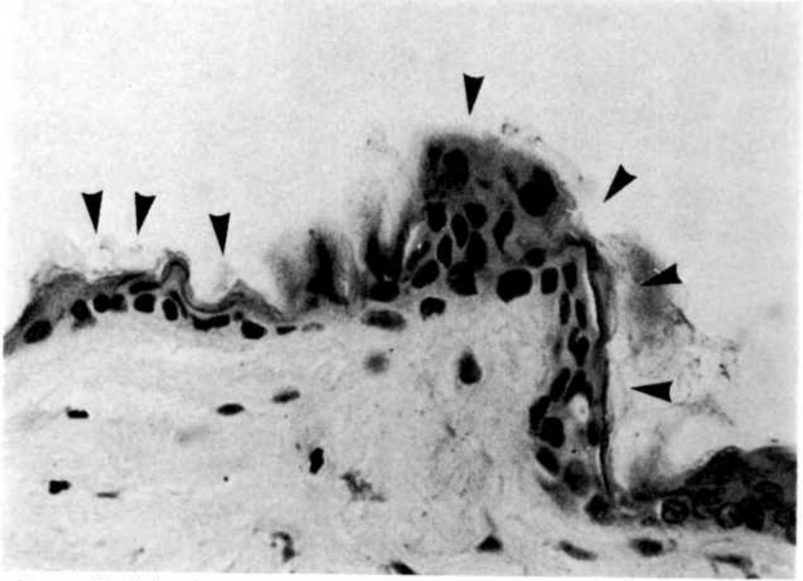

FIG. 2.—Focus of cellular degeneration and necrosis (arrowed) in mouse epidermis 1 hr after treatment with 10% sodium lauryl sulphate (H. and E. × 300).

mouse skins treated daily with 1% or 2·5% solutions of the surfactants is given in Table II. Treatment with 1% sodium lauryl sulphate resulted in an appearance of erythema after about 8 or 9 applications, whereas, when 2·5% was applied, the skin became thicker than normal and erythematous after 6 or 7 days. After 9 days the erythema was marked. In comparison, the changes produced by cetrimide were more severe and appeared earlier. Treatment with 1% cetrimide produced erythema and epidermal thickening as early as 3–4 days and after 6 or 7 days, scaling of the cornified layer was also present. At 2·5% the changes

TABLE II.—*The Time of Appearance of Visual Changes in Mouse Skins Treated with Daily Applications of Sodium Lauryl Sulphate, Cetrimide or Tween-80*

Treatment	Visual changes* (days) 1	2	3	4	5	6	7	8	9
Sodium lauryl sulphate									
1·0%	—	—	—	—	—	—	—	±	+
2·5%	—	—	—	—	—	—	±	+	+ +
Cetrimide									
1·0%	—	—	±	+	+	+ +	+ +	+ +	+ + +
2·5%	—	±	+	+	+ +	+ +	+ +	+ + +	+ + +
Tween-80									
1·0%	—	—	—	—	—	—	—	—	—
2·5%	—	—	—	—	—	—	—	—	—

* ± Slight oedema.
+ Moderate oedema and erythema.
+ + Oedema erythema and slight scaling.
+ + + Oedema profound, erythema and advanced scaling involving entire area of treatment.

TABLE III.—*Histological Changes in Mouse Skins Treated Daily with Applications of Sodium Lauryl Sulphate or Cetrimide*

	Concentration					
	1%			2·5%		
	Day					
Treatment	2	5	9	2	5	9
Sodium lauryl sulphate						
Hyperkeratosis	—	±*	+	± (1/5)	+	+
Parakeratosis	—	±	+ +	—	+	+ +
Intracellular oedema	—	+	+ +	—	+ +	+ +
Acanthosis	—	±	+	—	±	+
Ulceration and necrosis	—	—	—	—	—	—
Dermal cell infiltration	+	—	—	+	—	+ (2/5)
Cetrimide						
Hyperkeratosis	+	+ +	+ + +	+	+ +	+ + +
Parakeratosis	—	+	+ +	+ (2/5)	+ +	+ + +
Intracellular oedema	—	+ +	+ +	—	+ +	+ +
Acanthosis	—	+	+ +	—	+ +	+ + +
Ulceration and necrosis	—	—	—	+ (1/5)	+ (2/5)	+ +
Dermal cell infiltration	+	+ +	+ +	+ +	+ +	+ +

* Lesion seen in all animals unless otherwise stated.
± Slight.
+ Moderate.
+ + Pronounced.
+ + + Severe.

were similar but appreciably more severe and earlier seen. Erythema, thickening and oedema were manifest after only 2 or 3 daily applications; and, by 5 days, the scaling was considerable. After 9 days this scaling involved the entire area of treatment, when erythema was also very pronounced. Repeated application of Tween-80 did not produce macroscopic changes at 1 or 2·5% even after 9 days.

The histological changes produced in mouse skins by repeated application of the surfactants is given in Table III.

(a) *Sodium lauryl sulphate.*—Following 2 days' treatment with sodium lauryl sulphate, no changes were seen with 1% while with 2·5%, the epidermal changes were minimal. In only a single animal of 5 given the 2·5% solution were the stratum corneum and stratum spinosum slightly thicker than normal. In the dermis, capillary engorgement and oedema were evident and in some areas the number of leucocytes was slightly increased.

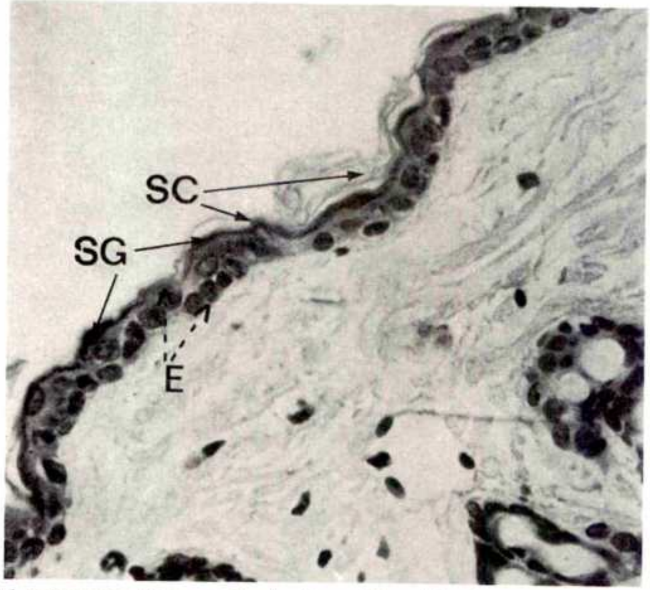

FIG. 3.—Normal epidermis of mouse back skin with minimal stratum corneum (SC), thin stratum granulosum (SG) and epidermal layer 1–3 cells across (E) (H. and E. × 300).

After 5 days' treatment with 2·5% sodium lauryl sulphate, the epidermal changes affected all animals and were more advanced than seen at 2 days.

The stratum corneum and stratum spinosum were appreciably thicker (Fig. 3, 4), and focal intracellular oedema was present. In the dermis capillary engorgement was more pronounced and the oedema widespread. Similar, but less prominent changes were apparent at this stage in those skins treated with 1% sodium lauryl sulphate.

Nine days' treatment with 1% or 2·5% sodium lauryl sulphate resulted not only in further increases in the thickness of the stratum corneum and an occasional appearance of nuclear debris in the keratin layer but also in a marked increase in the number of epidermal cells exhibiting intracellular oedema. Approximately 80% of the cells were affected. Numerous foci of cellular degeneration and necrosis were also evident at this stage. In the dermis the changes seen after 9 days were of a similar type to those identified after 5 days.

(b) *Cetrimide.*—The histological changes due to 1% or 2·5% cetrimide were much more severe than those produced by sodium lauryl sulphate. In most animals treated with cetrimide (1% or 2·5%) for 2 days, a moderate to marked dermal infiltration of leucocytes and mast cells was present and, in addition, some oedema and capillary engorgement occurred, although at this stage it was not widespread.

After 5 days' treatment with 1% or 2·5% cetrimide, the stratum corneum was 2–3 times the normal thickness and when stained with eosin or the PAS-reaction, the keratin appeared to be stratified. Alternate layers parallel to the skin surface stained lightly and darkly with these agents (Fig. 5). In the pale staining zones some nuclear debris was frequently evident (Fig. 6). Where this nuclear debris was most pronounced, the underlying cell layers of the epidermis were often greatly reduced, such that the stratum granulosum was absent altogether and the stratum spinosum and basal cell layer reduced in some areas to a single layer of much flattened and degenerate cells. Occasionally even this layer was absent. In less severely affected areas the squamous cell layer was considerably thicker than normal and was acanthotic. In this acanthotic epidermis the basal cells could be clearly identified by their position and their cuboidal or low columnar shape, with prominent spherical nuclei. The spinous cells, however, did not resemble those normally seen in this region, but were progressively flatter toward the skin surface. In the stratum granulosum the cells were occasionally

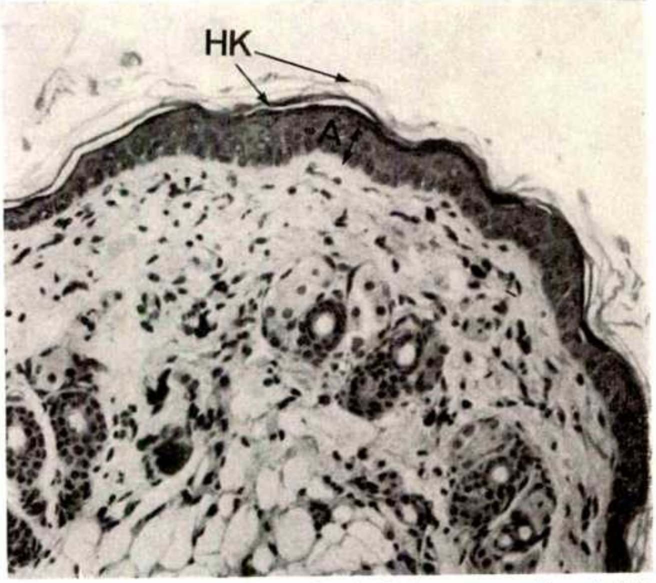

FIG. 4.—Hyperkeratinization (HK) and acanthosis (A) in mouse epidermis following 9 daily applications of 2·5% sodium lauryl sulphate (H. and E. × 180).

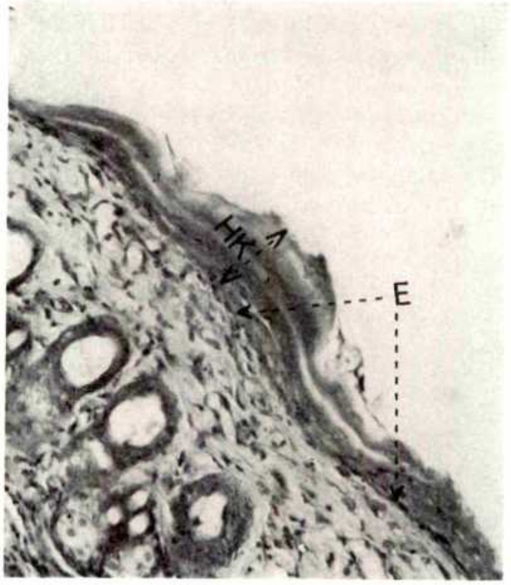

FIG. 5.—Hyperkeratinization (HK) with stratification of the stratum corneum in mouse epidermis following 9 daily applications of 2·5% cetrimide. The epidermal cell layer (E) is thinner than normal and the stratum granulosum absent (H. and E. × 250).

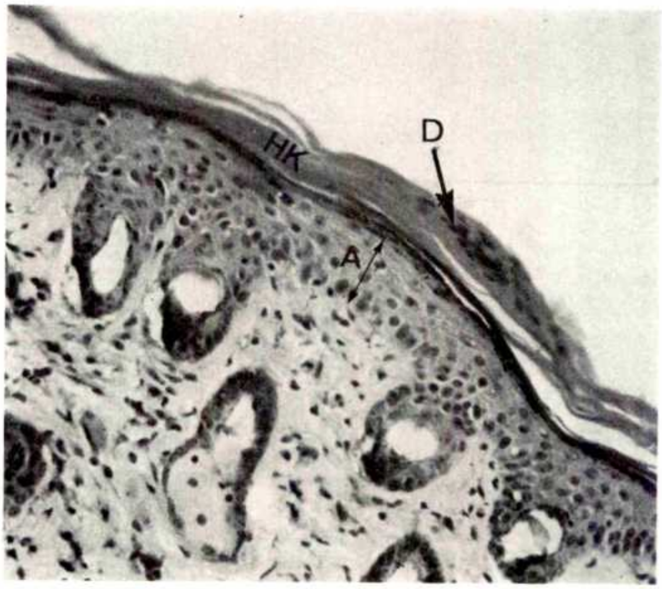

FIG. 6.—Hyperkeratinization (HK), nuclear debris (D) and acanthosis (A) in mouse epidermis following 9 daily applications of 1·0% cetrimide (H. and E. × 250).

more granular than normal and, in many instances, the layer was also wider than usual. Dermal changes in mouse skins treated with cetrimide for 5 days consisted of oedema, capillary engorgement and leucocyte infiltration, all of which were considerably more pronounced than after 2 days' treatment.

After 9 days the keratin layer was thicker than that seen at day 5, and contained abundant nuclear debris. In general the epidermal cell layers were much reduced such that only a narrow cellular zone, consisting of 1 or 2 layers of degenerate cells separated the keratin layer from the dermal tissues. In the stratum corneum the stratification was very pronounced and the amount of nuclear debris incorporated in it considerable. In the more severely damaged skins many microabscesses occurred in the epidermis, and in the dermis the degree of leucocyte infiltration was considerable. In all skins examined after 9 days' treatment severe capillary engorgement was present.

(c) *Tween* 80.—No histological changes consistent with the treatment given occurred in mouse skins exposed to 1% or 2·5% Tween-80 for up to 9 days.

DISCUSSION

The results show that, of the 3 surface active agents studied, sodium lauryl sulphate and cetrimide were irritant to mouse skin at the concentrations used, while Tween-80 was not. They demonstrate also that the cutaneous response to single applications of high concentrations of sodium lauryl sulphate and cetrimide is different from that to repeated applications of low concentrations. With repeated treatments, the histological manifestation of tissue damage preceded changes which could be appreciated with the naked eye.

Previous studies indicate that sodium lauryl sulphate is a potent primary irritant to human and animal skin (Epstein *et al.*, 1939; Olson *et al.*, 1962; Carson and Oser, 1964; Gisslen and Magnusson, 1966) and that cationic surfactants such as cetrimide cause skin damage (Scala *et al.*, 1968; Cutler and Drobeck, 1970). The low irritation potential of non-ionic agents such as Tween-80 has also been appreciated previously (Harrold, 1959; Honeywell-Atlas Report; Treon, 1963; Gisslen and Magnusson, 1966).

In rabbits treated with sodium lauryl sulphate, scaling of the skin was observed 24 hours after a single application containing 150 mg/kg; but, after a dose of 1200 mg/kg, the skin appeared leathery (Carson and Oser, 1964). Ten applications of 1–25% sodium lauryl sulphate to rabbits in a period of 2 weeks caused changes varying from erythema to severe " burns " (Olson *et al.*, 1962). In a more detailed study the histological changes seen in guinea-pig skin 24 hours after a single application of 1% solution consisted of nuclear pyknosis, intracellular vacuolation, focal necrosis in the upper part of the stratum spinosum, and a dermal inflammatory cell infiltration. At a concentration of 5% total epidermal necrosis was seen after 24 hours (Gisslen and Magnusson, 1966). These findings are essentially similar to the changes seen in the mice treated with a single application of 10% solution for considerably shorter periods (i.e. 0·5–6 hours). However, in the guinea-pig experiments, wax epilation was used before the application of the surfactant and, as a result, acanthotic changes were reported, even in control animals. Although Gisslen and Magnusson (1966) reported degenerative changes in the dermal collagen after sodium lauryl sulphate treatment, no changes were seen in the mouse skins, even with a 10% concentration. This difference is possibly due to the inability of the surfactant to penetrate into the dermis in the short test period (2 hours). Presumably, if observations using this concentration had been made after a longer period of exposure, some degenerative changes in the dermal collagen might have occurred.

Little data seems to be available concerning the irritancy of cationic surfactants on animal skins (Cutler and Drobeck, 1970). A 1% solution of benzalkonium chloride (a quarternary ammonium cationic surfactant) was reported to alter the permeability of the skin (Scala *et al.*, 1968) and to cause damage in a similar way to sodium lauryl sulphate (Gisslen and Magnusson, 1966).

The stratification in the stratum corneum in animals treated repeatedly with cetrimide has not been reported previously and is difficult to explain. In normal skins, keratin stains slightly with the PAS-reaction and is eosinophilic. It is possible that the weak staining with eosin and the strong PAS-positive reaction represents an incomplete formation of the keratin molecule and the presence of excess glycoprotein. If this is so, it is probable that cetrimide interferes with normal keratin synthesis such that the resulting keratin differs in its composition from normal. Jarrett, *et al.*, (1959) have shown that, under conditions of accelerated keratin production, such as in parakeratosis, the chemical composition of keratin and its staining characteristics can differ from normal. Abnormal keratin formation in parakeratosis has been related previously to an acceleration in the process (Van Scott and Fleisch, 1954a, b; Magnus, 1956).

It is possible that, in mouse skins, the presence of parakeratosis and of abnormally stained keratin represent an acceleration of keratin formation as well as an impairment in its synthesis due to cetrimide treatment. At present the exact nature of this abnormal keratin formation is obscure, but will form the subject of further studies.

The role of physico-chemical factors.—In an attempt to explain the different degrees of irritancy of the 3 surface active agents on mouse skin the physico-chemical properties of the solutions were considered; these included the surface activity, the lipophylic property and the protein-binding ability; all of these have been shown to be of importance in determining the response of subcutaneous tissue to injection of surfactants (Gangolli *et al.*, 1967).

In the present experiments the surfactants were applied to the mouse skins at concentrations which exceeded those capable of inducing irreversible damage to the plasma membranes of mammalian cells. Although the damage to mammalian cells by surfactants has been extensively studied using erythrocytes suspended in isotonic saline (Ponder, 1947; Love, 1956; Hooghwinkle *et al.*, 1965), Gangolli (1969) has shown that erythrocytes react in a similar way to other mammalian cells, such as fibroblasts or myocytes. Furthermore, the damaging effect of a surfactant has been correlated with its surface pressure and 2 general principles have been suggested (Schulman *et al.*, 1955). These authors believed that, to damage cells, a surface active agent must be at a concentration which reduces the surface tension of water from 720 to 380 μN/cm^2 (i.e. it exerts a surface pressure of 340 μN/cm^2). In this case, damage is induced by simple detergency. Alternatively, by virtue of its lipophilic potential, a surfactant must be capable of reacting with 1 or more of the lipid components and of altering the properties of the cell membrane. Lysis by the latter mechanism may occur at concentrations which have a surface pressure of less than 340 μN/cm^2. In the present work, the surface pressure exerted by all concentrations of the 3 surfactants used was higher than 340 $\mu N/cm^2$. At the concentrations used all were capable of removing surface lipids from mouse skin. Thus the surface pressure and lipophilic character of the surfactants are unlikely to account for the difference in the ability of Tween-80, sodium lauryl sulphate and cetrimide to cause irritation.

It is possible that protein-binding ability may account for the effects seen. This is supported by the fact that sodium lauryl sulphate and cetrimide both react with proteins such as collagen and keratin (Harrold, 1959; Gustavson, 1950; Witter and Mink, 1958; Kligman, 1969), the former to denature them and the latter to bind to them to form chemical-protein complexes. As a result of such a reaction a change in the properties of the protein may occur which, in the case of keratin, may lead to an impairment in the barrier function of the skin. The observations that sodium lauryl sulphate or benzalkonium chloride (cationic surfactant) treatment made human skin more permeable to potassium and sodium ions (Scala *et al.*, 1968) support this view. Increase in the permeability of the skin to metal ions after a period of treatment is likely to be accompanied by increase in permeability to other substances such as surfactants themselves (Blank and Gould, 1959, 1961; Scala *et al.*, 1968) and the ability of a surfactant to react with epidermal keratin can thus be correlated with the occurrence of damage to underlying tissues. This is consistent with an observation made by Blank *et al.*, (1964) that, in order to produce primary tissue damage, an irritant must first penetrate the skin. Also, as Wood and Bettley (1971) recently pointed out, an important factor in the production of irritation is the ability of an agent to denature the keratin in such a way as to increase its permeability and enhance the penetration of the irritant. Some non-ionic surfactants such as Tween-80, do not increase the permeability of the skin (Bettley, 1961) and would not therefore be expected to penetrate the skin in significant amounts (Harrold, 1959). This low degree of penetrability is perhaps another major factor contributing to the differences observed in the skin changes between Tween-80 and the other 2 surfactants studied by us.

CONCLUSION

Our studies indicate that, of the physico-chemical properties possessed by the surfactants studied, surface activity and lipophilia do not contribute to the epidermal damage caused. On the other hand, the protein-binding ability is of primary significance. It is probable that the nature of the interaction of the surfactant with epidermal protein determines the extent and severity of the damage.

The type of epidermal response to single applications of high concentrations of surfactants differs considerably from that produced by repeated administration of lower concentrations. Since the latter treatment simulates more closely the actual conditions of use for cosmetics, e.g. soaps and creams, a detailed study of the changes produced may contribute to a better understanding of chronic non-allergic contact dermatitis produced by surfactants.

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LOCALIZED UNILATERAL HYPERHIDROSIS—A CLINICAL AND LABORATORY STUDY

W. J. CUNLIFFE, C. E. JOHNSON AND D. M. WILLIAMSON

Summary.—Three cases of unilateral idiopathic hyperhidrosis are described. Two of the lesions were congenital; the third occurred in later life. Quantitative sweat collections demonstrated that the sweat glands in the lesion were hyper-responsive to those stimuli, which normally evoked sweating in the skin sites where the lesions occurred. Hyper-responsiveness of the sweat glands to cholinergic stimuli was also demonstrated, and histology revealed greatly enlarged glands.

These results suggest that the hyperhidrosis is due to increased glandular mass, although sweat gland hypersensitivity could not be ruled out as an additional contributing factor to the hyperhidrosis.

Local anticholinergic therapy was found to be successful in the treatment of all 3 patients.

SYMMETRICAL idiopathic hyperhidrosis affecting either the palms, soles, face or axillae is not uncommon; but occasionally the hyperhidrosis is unilateral and may involve other skin sites.

This paper reports the investigation of 3 patients with localized hyperhidrosis occurring at unusual sites.

SUBJECTS

The clinical features of the 3 patients are shown in Table I, and Fig. 1 demonstrates the intensity of the sweating in Case 1. The attacks of sweating in Cases 1 and 2 rarely occurred more frequently than once a day, whereas the patient with the forehead lesions usually had several attacks daily. However, in all patients each episode lasted for 20–30 min.

TABLE I

Patient	Age and sex	Site	Duration
1	32 F	Dorsum of lower, right forearm, 6 × 4 cm	As long as she could remember
2	21 F	Dorsum of lower, right forearm, 8 × 5 cm	As long as she could remember
3	67 M	Right side of forehead, 3 × 3 cm	2 years

General examination of the patients revealed no further abnormalities, other than chronic bronchitis in Case 3, and this limited the number of investigations which could be done.

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METHODS

Sweating was induced by various stimuli and collected for timed periods into preweighed filter papers. Loss of sweat by evaporation was minimized by the use of 2 methods. The filter papers were either placed inside an unventilated capsule glued to the skin or were covered by polythene, gauze and waterproof plaster. Collections were made from the involved skin and from a symmetrically opposite area of skin.

The effect of indirect heat was investigated in all patients by immersing the legs in water at 45°C for 30 min, and in 2 patients the effect of direct heating was also assessed by immersing the forearms and hands in water at 45°C for 5 min.

The effect of mental activity was investigated by giving all the patients difficult problems in mental arithmetic during the period of sweat collection, and gustatory sweating was measured whilst eating a typically English, non-spiced, hospital meal.

Sweat gland responsiveness was investigated by measuring the sweat response to intradermal injections of 3 different doses of β-methacholine, each injection being given on different days.

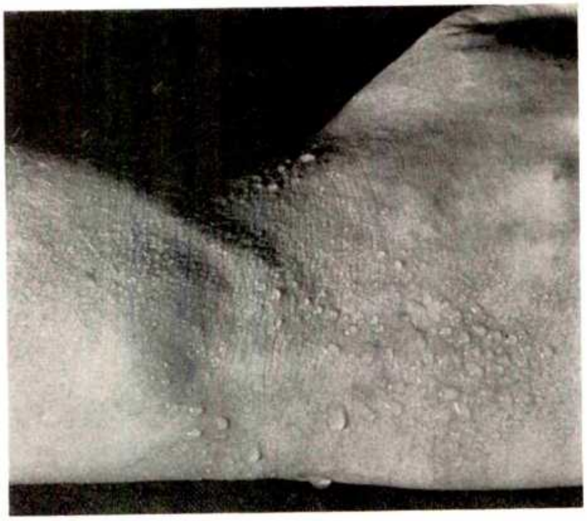

FIG. 1.—The severity of sweating.

Biopsies were taken from the affected and symmetrically opposite normal areas of skin in all 3 patients. The relative size of the sweat glands in the biopsies was noted and an attempt was made to estimate the sweat gland density. Cholinesterase activity was measured histochemically.

RESULTS

Data on the thermal, mental and gustatory stimuli are shown in Table II. Indirect heat produced a considerably increased sweat response on the abnormal side in all patients; direct heat was studied only in the patients with the limb lesions and, compared to the normal side, the lesions produced an excessive response. In Case 2 (Table III) direct heating was repeated twice at 30 min intervals; after an initial 5-min exposure, no further excessive hyperhidrotic response could be elicited within 1 hour. However, on re-testing 2 days later, a grossly abnormal response was again obtained.

Gustatory sweating was only measured in the patient with the forehead lesions,

TABLE II

Stimulus	Patient	Sweat rate (mg/cm²/h) Normal side	Sweat rate (mg/cm²/h) Affected side
Indirect heat	1	12·7	172·2
	2	25·2	86·0
	3	13·9	43·9
Direct heat	1	4·8	85·6
	2	2·9	133·3
	3	—	—
Food	1	—	—
	2	—	—
	3	0·0	22·3
Mental arithmetic	1	0·0	0·5
	2	0·9	0·0
	3	—	—

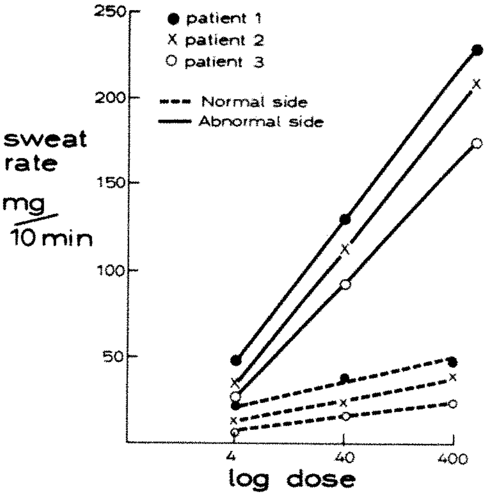

FIG. 2.—The dose response curve to intradermal β-methacholine.

TABLE III.—*The Effect of Repeated Direct Heating on the Hyperhidrosis in Patient 2*

	Time of heating	Sweat rate (mg/cm²/h) Normal	Sweat rate (mg/cm²/h) Affected
Day 1	0–5 min	7·7	210·0
	30–35 min	8·7	19·0
	45–50 min	17·0	20·6
Day 3	0–5 min	7·7	296·0

and he produced much more sweat on the affected side. This patient similarly was the only subject to develop an abnormal and increased sweat rate with mental stress.

The dose response curves to β-methacholine are similar in all 3 patients (Fig. 2); the abnormal side produced much more sweat for each dose of β-methacholine in comparison with the control side.

The skin from the hyperhidrotic skin showed greatly enlarged sweat glands in all 3 patients. It was not possible to assess sweat gland density accurately from the serial sections, and there was no detectable differences in the cholinesterase activity between the normal and hyperhidrotic skin.

However, all patients have responded to the topical anticholinergic 5% poldine methanesulphate (" Nacton ") in talc or 70% alcohol, applied twice a day (Table IV).

TABLE IV.—*Effect of Topical Poldine Methosulphate on Sweating (After Direct Heating)*

	Sweat rate (mg/cm²/h)	
Patient	Before treatment	After treatment
1	85·6	2·1
2	133·2	3·4

DISCUSSION

Unilateral idiopathic hyperhidrosis is a rare condition; facial lesions affecting either the right or left side of the face have been reported by Tarlov and Herz (1947), Tankel (1951), Pearce (1964) and Shafer (1966). The sweat glands were judged to be hypersensitive to cholinergic drugs, but histological examination of the sweat glands was not performed. Mellinkoff and Mellinkoff (1950) and Mellinkoff (1951) reported 2 lesions of the knees and wrist which showed gustatory sweating, but in these cases hypersensitivity to cholinergic drugs was not found; no histological examination of the sweat glands was done. In only 1 patient have the sweat glands been examined histologically. Lapière (1957) described grossly enlarged glands in a lesion occurring on the elbow; this was also associated with a superficial pigmentary naevus.

From all these reports it appears that there are 2 types of lesion, 1 where the sweat glands show normal sensitivity to cholinergic drugs (Mellinkoff and Mellinkoff, 1950; Mellinkoff, 1951) and the other where increased sensitivity was noted (all other reports). Our 3 patients seem to belong to the latter group.

Histological examination of the sweat glands of the lesions in each of our patients showed increased size of the glands. This is in agreement with the findings of Lapière (1957). The increased size of the sweat glands alone might account for the excessive sweating, but sweat gland hypersensitivity cannot be ruled out as a contributory factor. These lesions, therefore, may either be variants of the pure anatomical naevi commonly found in the skin or may have arisen originally as functional naevi which showed secondary hypertrophy of the glandular elements. An example of a pure functional naevus has recently been described (Greaves *et al.*, 1970).

Heat precipitated the hyperhidrosis in all of the 3 patients, but Case 3 alone showed excessive sweating to gustatory and mental stimuli. These differences may be due simply to the type of innervation which the dorsum of the hand and the forehead receive. The normal sweat glands of the face respond to heat, gustatory influences and mental activity, while the glands in the dorsum of the hand respond to heat alone.

A finding of interest was the apparent ease with which the sweat glands became refractory to stimulation in Case 2 (Table II). Sweat gland fatigue is well documented, and it generally occurs after several hours' sweating. Sarkany *et al.*

(1965) have shown that fatigue is due not to glandular exhaustion but to the hydration of the stratum corneum producing mechanical obstruction to the exit of sweat. With the excessive sweating seen in this patient 30 min may be sufficient to produce the necessary degree of hydration to reduce the sweat rate so dramatically. However, true glandular fatigue may have occurred, in which case the glands would be highly atypical.

Asymmetrical localized areas of hyperhidrosis have been shown to occur with lesions involving any part of the sympathetic nerve pathway such as syringomyelia. Certain localized areas of hyperhidrosis may also occur after stellate ganglionectomy; this is thought to be due to nervous pathways developed from epibranchial placodes (Monro, 1959), but such hyperhidrotic areas are normally found on the face. Our patients clearly do not fall into these latter 2 groups; we feel that our cases represent unusually localized, perhaps functional, sweat gland lesions, 2 of which are obviously congenital in origin.

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PROGRESSIVE CHANGES IN CUTICULAR PATTERN ALONG THE SHAFTS OF HUMAN HAIR AS SEEN BY SCANNING ELECTRON MICROSCOPY*

EVA BOTTOMS, EDWARD WYATT AND STANLEY COMAISH

Summary.—A study of normal hair shafts by scanning electron microscopy shows that there is a regular and progressive loss of the normal cuticular scale pattern from root to tip. Appearances found in pathological conditions should be interpreted in the light of this normal variation.

ON examining normal hair by scanning electron microscopy (SEM) we have observed that the shaft nearest the root nearly always has regularly edged cuticular scales (Fig. 1), whilst that further from the root shows crenellated edges (Fig. 2). Towards the end of long head hairs there is increasing loss of cuticular scale (Fig. 3), and at the tip (Fig. 4) the pattern is almost completely absent so that the fusiform cells of the cortex are exposed. Fig. 1–4 are from a single hair of a male aged 19.

To assess the extent of these deviations from the regular pattern, scalp hairs were taken from the vertex of 19 women and 9 men aged 18–52 not known to be suffering from any disease. Single hairs were plucked quickly to minimize trauma (McGuire and Kligman, 1964), and from each subject 2 hairs with macroscopically intact roots were laid on to graph paper so that 3 mm segments at known intervals from the root could be obtained. Specimens of hair 24 years and 18 years old were also studied. These came from samples which had been kept from the first hair cut of a boy and girl aged 4 and 3 respectively. The hair segments were not prepared in any way, but placed on to SEM stubs and then gold coated.

All specimens were examined in a standard manner, using a Stereoscan Mark II microscope, and were tilted at 45° to the beam. Photographs were taken of the mid-point of several 3 mm segments of each hair at × 1000 magnification setting. To assess the changes in the appearance of the hair a scheme of scoring was used (Table I). The photographs were read blind in a random order. On repeating the reading there was a different assessment in 10% of the photographs, and this by only 1 degree of change; this was not significant ($P > 0{\cdot}05$).

The overall tendency is indicated in Fig. 5; individual hair shafts invariably showed progressive loss of the normal cuticular scale pattern from the root to the tip. Eventually, when it is long enough, a hair loses all pattern although minor changes from a regular pattern may be present very close to the root.

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* Based on a paper read at a meeting of the Scottish Dermatological Society, Dundee, June 25th, 1971.

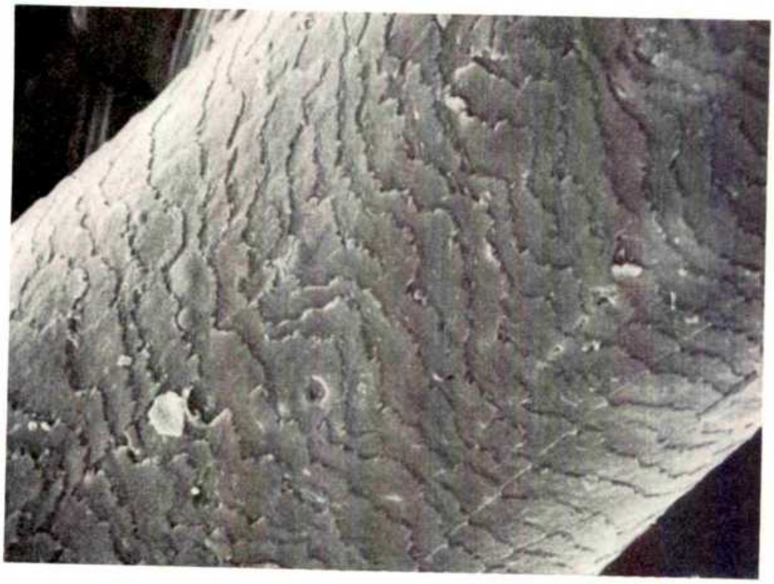

FIG. 2.—207 mm from root (× 860).

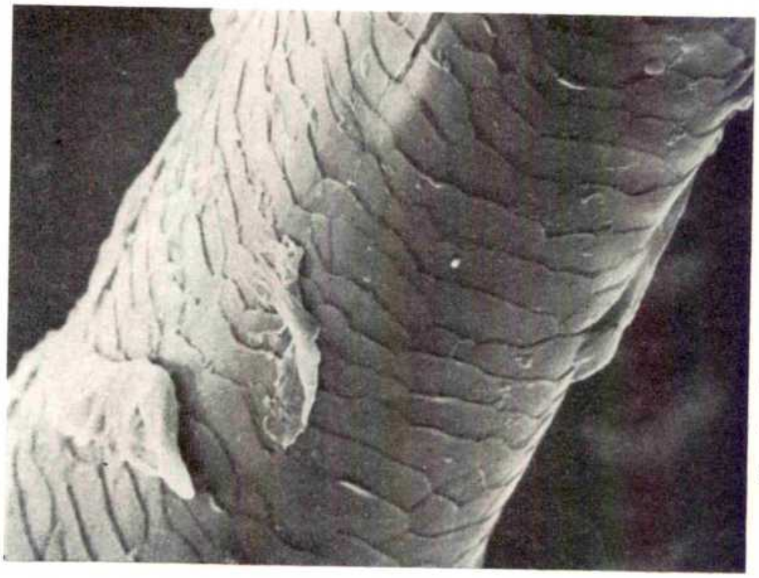

FIG. 1.—Two mm from root (× 1000).

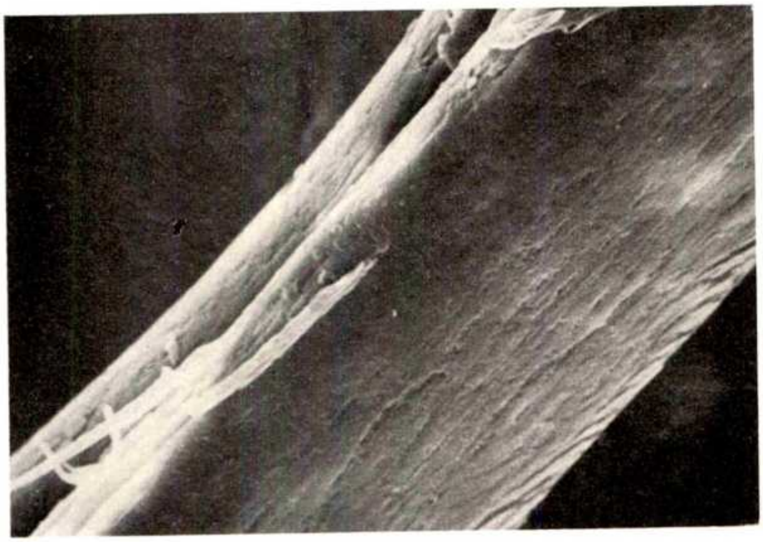

Fig. 4.—Tip of hair, 299 mm from root (× 860).

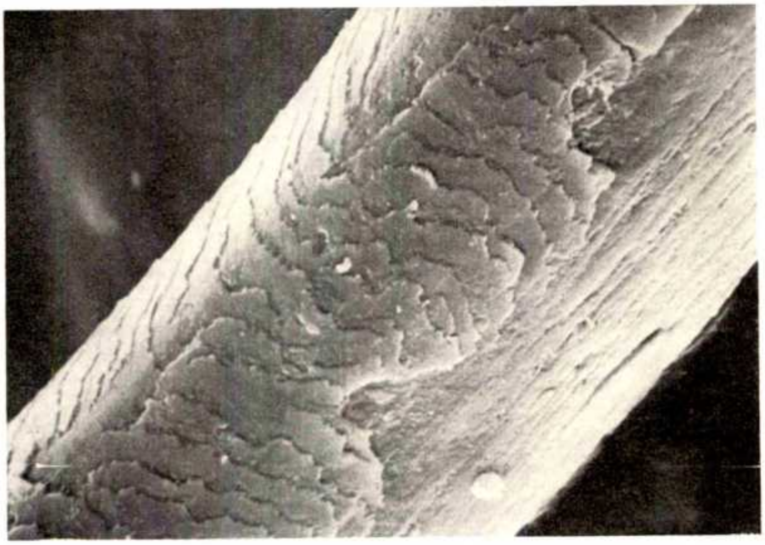

Fig. 3.—266 mm from root (× 880).

TABLE I

Appearance of hair shaft	Score
All cuticular scales regular	0
1–2 cuticular scales crenellated	1
3–6 cuticular scales crenellated	2
7 or more cuticular scales crenellated	3
All cuticular scales crenellated	4
All cuticular scales crenellated plus 1–2 scales absent	5
All cuticular scales crenellated plus 3–6 scales absent	6
All cuticular scales crenellated plus 7 or more scales absent	7
Complete loss of pattern	8

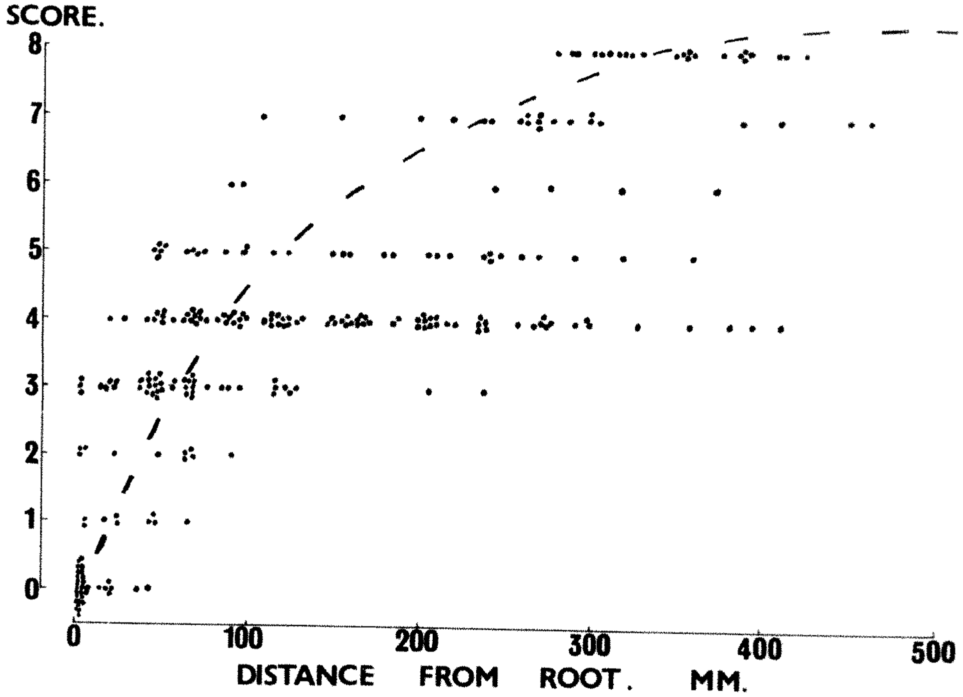

FIG. 5.—Relationship between hair shaft appearance and distance along shaft.

Hair is daily subject to physical and chemical trauma as well as to the effects of ageing. The proximal part of stored old hair (Fig. 6) shows a crenellated cuticular scale pattern, whilst the distal tip (Fig. 7) shows complete loss of pattern. Although the precise site of cutting of this hair is unknown, it is apparent that cuticular pattern may be retained proximally for at least 24 years, and that ageing *per se*, with its accompanying changes such as dehydration, does not obliterate it. Thus the cuticular variation would seem likely to be related to the amount of trauma from daily brushing, combing and clothing friction to which hair is subject.

Scanning electron microscopy is being increasingly used to study hair changes in pathological conditions (Orfanos *et al.*, 1970; Caputo and Ceccarelli, 1969; Dawber and Comaish, 1970; Mahrle *et al.*, 1969). The present observations

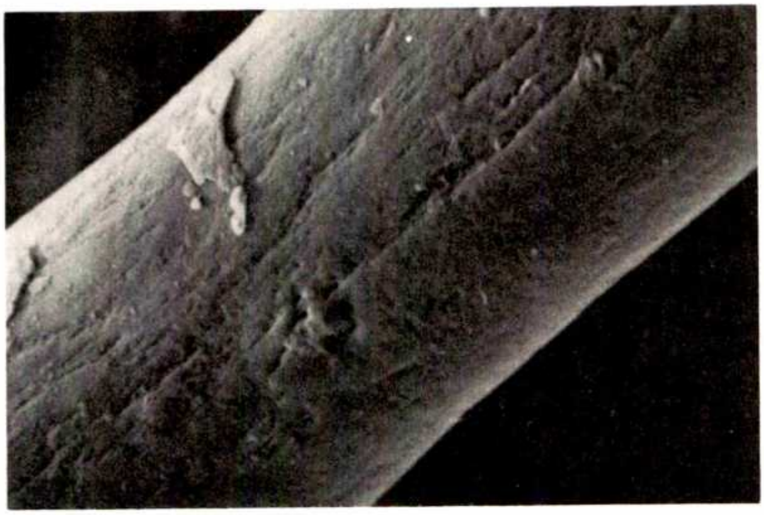

FIG. 7.—Distal tip 18 year old hair (× 1100).

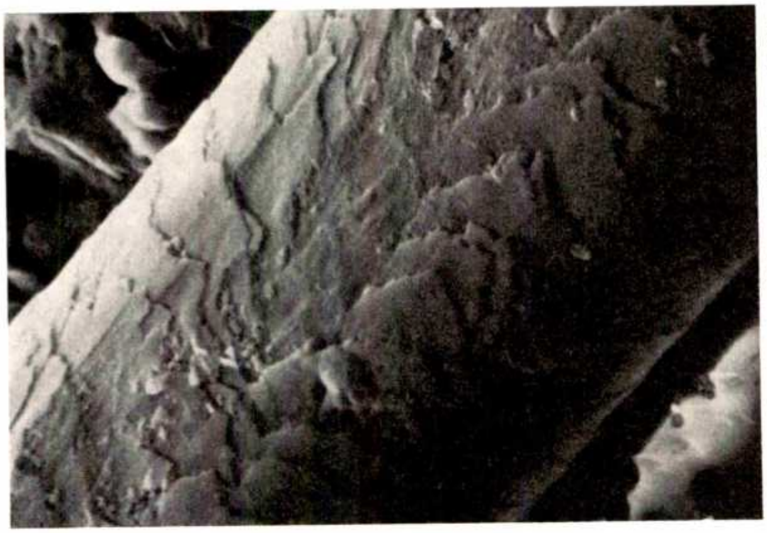

FIG. 6.—Proximal end 18 year old hair (× 1100).

indicate the importance of interpreting findings in the light of the normal variation of the cuticular scale pattern from root to tip described in this paper.

Mr E. M. Boult, the School of Chemistry, University of Newcastle upon Tyne, provided facilities for this study.

We wish to thank Professor Sam Shuster for his advice and Mr H. Sargent for invaluable technical assistance.

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INTRACELLULAR LOCALIZATION OF TYROSINASE INHIBITOR IN AMELANOTIC AND MELANOTIC MALIGNANT MELANOMA

TOSHIO HAMADA AND YUTAKA MISHIMA

Summary.—Intracellular distribution of the tyrosinase inhibitor in malignant melanoma cells has been investigated in order to elucidate the defect of melanization in the pigment cell cytoplasm and premelanosomes of amelanotic melanoma. We have found that the tyrosinase inhibitor has its highest activity in the cytoplasmic soluble fraction for both amelanotic and melanotic melanomata. However, inhibitor assay of melanoma subfractions reveals a distinct difference between the inhibitor activity of amelanotic and melanotic melanomata. Premelanosome-containing subfractions of amelanotic melanomata contain much higher inhibitor activity than those of melanotic malignant melanomata. A high concentration of inhibitor seen in subfraction 3 which contains most of the total succinoxidase activity is found to be contained in the premelanosomes of this subfraction.

It is suggested that the absence of melanization in the non-premelanosomal cytoplasm of pigment cells and in the premelanosomes of tyrosinase containing amelanotic melanomata is directly related to the concentration of tyrosinase inhibitor in these compartments of the cells.

MELANOGENIC control of the pigment cell is thought to be dependent on both tyrosinase synthesis in ribosomes and premelanosomal tyrosinase activity (Seiji *et al.*, 1963; Mishima *et al.*, 1971). However, there appears to be a subcellular control mechanism of melanogenesis independent of the level of production and cytoplasmic transfer of tyrosinase. Biochemical properties of naturally-occurring inhibitors of melanin synthesis have been described in malignant melanomata and in pigmented and non-pigmented skin (Satoh and Mishima, 1967, 1969; Chian and Wilgram, 1967). Despite the recent clarification of the chemical properties of these melanogenic inhibitors (Satoh and Mishima, 1970; Flawn and Wilde, 1970; Flawn *et al.*, 1970), their role and the intracellular distribution of their activity are not yet known. Therefore we have studied these problems, using Fortner's melanotic malignant melanoma, which is actively engaged in melanin synthesis, as well as its amelanotic counterpart, which represents interrupted melanogenesis.

MATERIALS AND METHODS

Fortner's amelanotic malignant melanoma type 3 (AM-3) and Fortner's melanotic malignant melanoma type 1 (MM-1) were obtained from Joseph G. Fortner, Sloan Kettering Institute, New York, and transplanted thereafter in our laboratory. AM-3 had been preserved by Fortner in frozen storage prior to our transplantation.

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Syrian (golden) hamsters used for transplantation were obtained from the Lakeview Hamster Colony, Newfield, New Jersey, and were the same strain as that used by Fortner.

Method of Inhibition Study

(a) *Preparation of tissues for tyrosinase inhibitor assay.*—Non-necrotic melanomata, excluding epidermis, were homogenized in 0·25 mol/l sucrose using a Teflon head hand homogenizer. Density gradient fractionation of the homogenates was carried out as reported previously (Mishima, 1966), together with electron microscopy, following a procedure modified after Seiji *et al.* (1963).

(b) *Assay of tyrosinase inhibitor.*—Assay of tyrosinase inhibitory activity of fractions and subfractions was carried out in triplicate by measuring the extent of inhibition of partially purified mushroom tyrosinase by these fractions, since the inhibitor is found to have a similar and parallel effect on both mushroom and mammalian melanoma tyrosinase (Satoh and Mishima, 1967). The extent of inhibition by the addition of samples is expressed as % with respect to the tyrosinase activity of the control taken as 100%. Tyrosinase assay was carried out in triplicate in 1 cm cells with a Beckman spectrophotometer, Model DU. The initial rate of linear increase in optical density at 475 mμ, based on the formation of dopachrome, was measured, using L-3,4-dihydroxyphenylalanine (L-dopa) as substrate. The final reaction mixture, 6 ml contained 0·5 μmol/ml of L-dopa, mushroom tyrosinase (Sigma Chem., 900–1500 units/mg) 0·1 mg per 3 ml, and 35 μmol/ml of phosphate buffer at pH 6·8. The total protein of each fraction was measured by the biuret method. In those assays in which tyrosinase was to be inactivated the sample was boiled for 10 min, since the tyrosinase inhibitor (Satoh and Mishima, 1967) is heat stable, as reported previously.

(c) *Deoxycholate treatment.*—Sodium deoxycholate was added to the homogenate in 0·25 mol/l sucrose to obtain a final concentration of 0·4% The homogenate was kept at room temperature for 30 min and, following this, the fractionations and inhibitor assays were performed.

(d) *Succinoxidase.*—The activity was determined according to the technique described by Potter (1964).

(e) *L-dopa preincubation method.*—Preincubation of the large granule fractions in L-dopa was carried out as described by Ishikawa *et al.* (1966).

(f) *Electron microscopy.*—For electron microscopy, aliquots of each subfraction were mixed at 4°C with 2% osmium tetroxide of Millonig and centrifuged at 105,000 *g* for 1 hour. The sediment obtained was then dehydrated in a graded series of ethyl alcohols and embedded in Maraglass 655. Electron microscopic preparation and dopa reaction of melanoma tissues used in this study were carried out following procedures described elsewhere (Mishima *et al.*, 1971). Sections were stained with uranyl acetate and Reynold's lead citrate and examined with an RCA EMU-3G electron microscope.

RESULTS

Tyrosinase inhibitor activity has been demonstrated in the soluble fraction and also in the large and small granule fractions of amelanotic and melanotic Fortner's melanomata. Inhibition of tyrosinase activity shows a linear increase proportional to the amount of inhibitor obtained from increments of melanoma tissue (Fig. 1). It is found that tyrosinase inhibitor is highest in the cytoplasmic soluble fractions from both amelanotic and melanotic melanoma, having approximately double the activity of any of the other fractions (Fig. 2). Among the remaining fractions the concentration of tyrosinase inhibitor from amelanotic melanoma is higher in the large granule than in the small granule fraction. This distribution is, however, reversed in melanotic melanoma. Electron microscopy of these fractions reveals that the large granule fraction consists of mitochondria, premelanosomes and melanosomes, while the small granule fraction contains chiefly the ribosomes. The intracellular distribution of the inhibitor obtained by this fractionation is re-examined before and after deoxycholate treatment in order to determine the extent of the confinement of the inhibitor to the membranes of the large and small granules. It is found that the distribution of tyrosinase inhibitor is unchanged after destruction of the membranes with deoxycholate.

In order to study the concentration of tyrosinase inhibitor in premelanosomes and other organelles, the large granule fraction of both melanomata is further subfractionated by gradient ultra-centrifugation to obtain separation of various stages, I–IV (Fitzpatrick), of melanosome formation and mitochondria. The characteristics of these 6 subfractions with respect to their activity for the melanogenic enzyme tyrosinase and the mitochondrial enzyme succinoxidase are summarized in Table I and II. The assay of tyrosinase inhibitor among these 6 subfractions is then carried out (Table I and II, Fig. 3). Large granule subfractions 4, 5, 6, containing various stages of melanosome formation, exhibit distinctly higher activity of tyrosinase inhibitor (750%, 440%, 290%, respectively) in the amelanotic than in the melanotic melanomata.

The large granule subfraction 3 has been found to contain the highest activity among the subfractions for both amelanotic and melanotic melanomata. This subfraction 3 is found to contain almost all of the total mitochondrial enzyme succinoxidase of the 6 subfractions, indicating that the mitochondria are concentrated in this subfraction. On the other hand, the tyrosinase activity is more widely distributed among the subfractions 3 to 6 corresponding to the known spectrum of melanosome maturation and tyrosinase distribution. Thus sub-

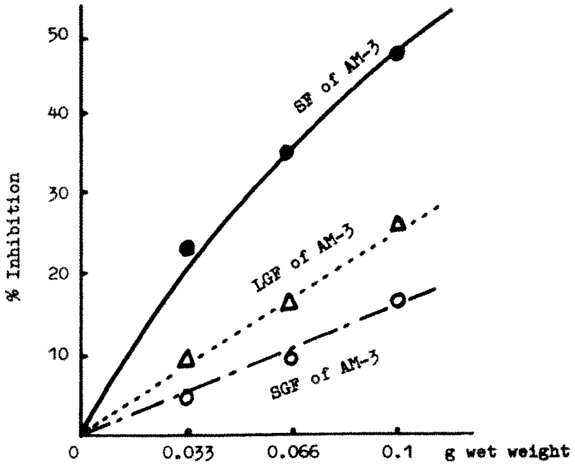

FIG. 1.—The tyrosinase inhibitor activity revealed by the inhibition of mushroom tyrosinase. There is a linear increase of inhibitory activity proportional to the amount of inhibitor obtained from increment of melanoma tissue.

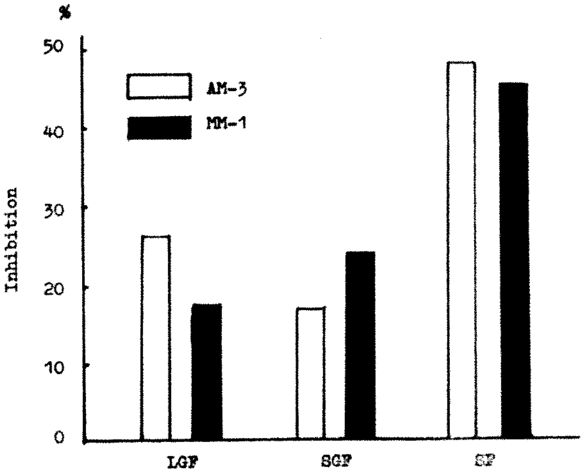

Fig. 2.—Intracellular distribution of the tyrosinase inhibitor among large granule, small granule and soluble fractions of Fortner's amelanotic and melanotic melanomata.

fraction 3 is examined under the electron microscope. A preponderance of mitochondria is revealed, although there is also a number of premelanosomes which appear to be smaller and less melanized than those of subfractions 5 and 6 (Fig. 4, 5). In order to determine further the sites of inhibitor distribution among the organelles of subfraction 3, electron microscopy and inhibitor assay are carried out before and after dopa incubation, which separates premelanosomes, by their induced melanization, from the mitochondria. Electron microscopy shows an increase in the electron density of the remaining small premelanosomes, but not in the mitochondria (Fig. 6). The organelle composition of Fortner's melanoma cells *in situ* with and without dopa preincubation is shown in Fig. 7, 8. Tyrosinase inhibitor assay of the large granule subfractions of dopa-incubated melanotic

TABLE I.—*Distribution of Premelanosomal and Mitochondrial Enzymes, and Tyrosinase Inhibitor (Melanotic Melanoma)*

	Inhibitor assay			Tyrosinase		Succinoxidase	
Large granule fractions	mg Protein/ 0·1 g wet tumour	Inhibition (%)/0·1 g wet tumour	Inhibition (%)/mg protein	μmol dopa converted/ h/g wet tumour	μmol dopa converted/ h/mg protein	$\mu IO_2/h/$ 0·1 g wet tumour	$\mu IO_2/h/mg$ protein
1	0·24	2·8	12	0·36	0·15	2	12
2	0·27	4·3	16	0·92	0·34	19	60
3	0·99	17·7	18	3·10	0·36	114	139
4	0·62	1·7	3	1·18	0·22	26	53
5	0·50	3·4	7	3·17	0·74	6	12
6	0·31	1·4	5	0·42	0·18	5	13

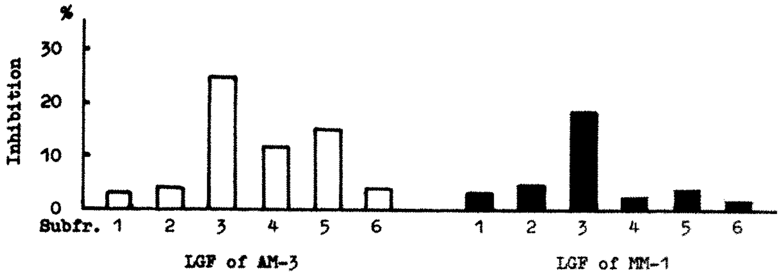

Fig. 3.—Intracellular distribution of the tyrosinase inhibitor among subfractions of the large granule fraction (LGF) obtained from Fortner's amelanotic and melanotic melanomata.

TABLE II.—*Distribution of Premelanosomal and Mitochondrial Enzymes, and Tyrosinase Inhibitor (Amelanotic Melanoma)*

	Inhibitor assay			Succinoxidase	
Large granule fractions	mg Protein/0·1 g wet tumour	Inhibition (%)/0·1 g wet tumour	Inhibition (%)/mg protein	$\mu IO_2/h/0\cdot1$ g wet tumour	$\mu IO_2/h/mg$ protein
1	0·24	3·5	15	4	19
2	0·26	4·7	18	21	63
3	0·89	25·8	29	123	134
4	0·51	12·8	25	34	68
5	0·34	15·0	44	11	31
6	0·24	4·0	17	6	19

melanoma, showing heavily melanized premelanosomes by electron microscopy, reveals that there is a shift in the distribution of inhibitor from subfractions 3 to 4, 5, and 6 after the incubation (Fig. 7). This indicates a substantial portion of the inhibitor-containing organelles of subfraction 3 in premelanosomes.

DISCUSSION

The implication of the present findings on tyrosinase inhibitor is 2-fold. Although tyrosinase activity has been demonstrated in pigment cell cytoplasm (Mishima *et al.*, 1971; Mishima, 1966; Mishima and Adams, 1969) there is, as yet, no explanation for the absence of melanization outside the premelanosome. In addition, the melanogenic defect (Mishima and Cooper, 1966) leading to the absence of melanization in tyrosinase-containing amelanotic melanomata has not been clarified. The presence and chemical properties of melanogenic inhibitor in pigment cell cytoplasm and melanoma cells has been described by many workers (Satoh and Mishima, 1967, 1969, 1970; Chian and Wilgram, 1967; Flawn and Wilde, 1970; Flawn *et al.*, 1970).

We have previously found that our inhibitor is a heat stable, dialysable, competitive tyrosinase inhibitor (Satoh and Mishima, 1967) which does not exhibit sulphydryl properties (Satoh and Mishima, 1967) but is substantially inactivated by u.v. irradiation (Hamada and Mishima, in preparation). This inhibitor has been further shown by column and paper chromatography to be a peptide composed of 6 amino-acids for melanotic, and 8 amino-acids for amelanotic, melanomata of Fortner (Satoh and Mishima, 1970).

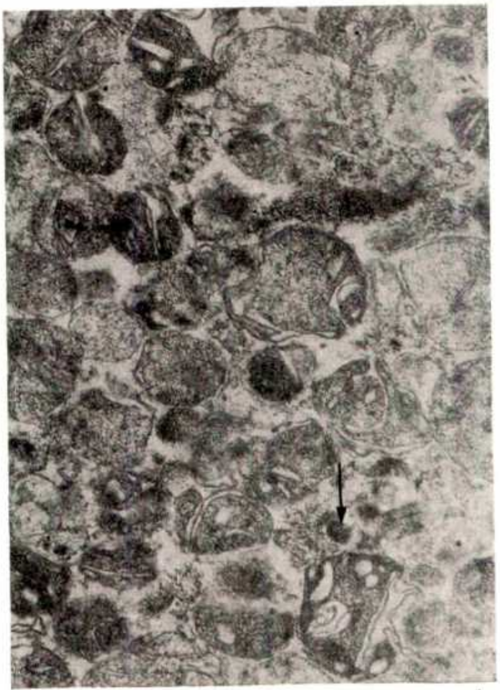

FIG. 4.—Electron micrograph of large granule subfraction 3 obtained from Fortner's amelanotic melanoma containing a large number of mitochondria and a few suspected premelanosomes (←). (×25,000.)

It is found that our tyrosinase inhibitor is active against mammalian melanoma tyrosinase as well as against mushroom tyrosinase (Satoh and Mishima, 1967; Hamad and Mishima, in preparation). Yasunobu (1959) has shown that various tyrosine-containing peptides may act as substrates for mushroom tyrosinase, but those peptides containing more than 2 peptide bonds are unable to act as substrates or competitive inhibitors for mammalian tyrosinase, possibly because of steric hindrance of the enzyme.

Previous studies dealt only with certain supernatants of tissue homogenate and were not directed towards determining intracellular distribution of melanogenic inhibitors. On the other hand it has already been established by electron microscopy and gradient biochemistry that tyrosinase is synthesized by ribosomes and

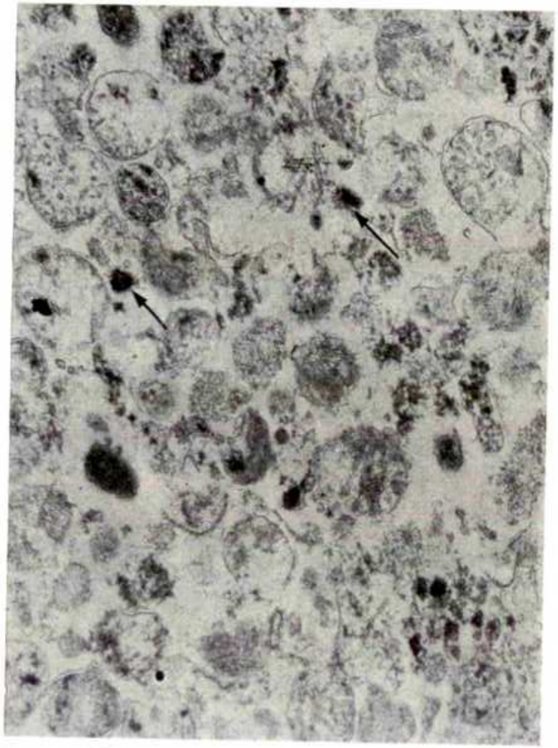

FIG. 5.—Electron micrograph of large granule subfraction 3 obtained from Fortner's melanotic melanoma, exhibiting mitochondria and a number of distinctly visible premelanosomes (←). (× 15,500.)

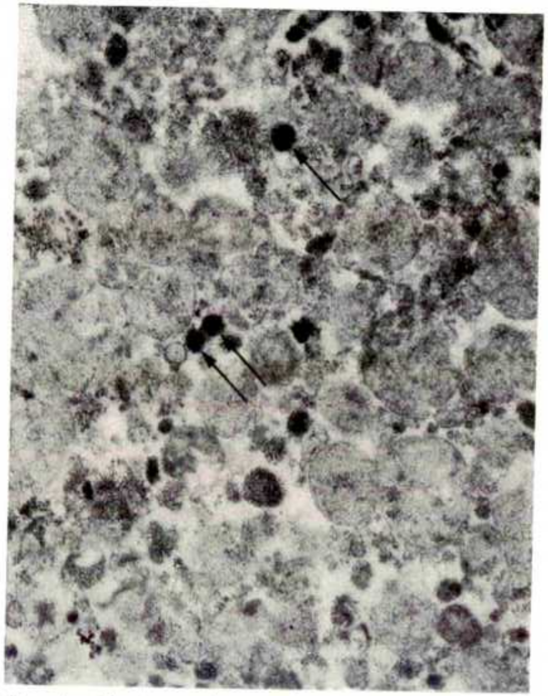

FIG. 6.—Electron micrograph of large granule subfraction 3 of Fortner's melanotic melanoma after dopa incubation, revealing newly formed dopa melanin within the premelanosomes (←). (× 16,000.)

then transferred to the premelanosomes formed by the Golgi apparatus, at which point melanization can proceed. Despite the absence of non-premelanosomal melanization *in vivo*, the predominace of tyrosinase in the soluble fraction of Fortner's melanotic and amelanotic melanoma can be demonstrated by biochemical assay using manometric (Mishima and Johnson, 1968), spectrophotometric (Satoh and Mishima, 1969) and ^{14}C-tyrosine conversion methods (Mishima *et al.* 1971). In agreement with these biochemical assays electron microscopic dopa reaction of Fortner's melanotic melanoma cells can reveal newly-synthesized dopa-melanin, not only within premelanosomes and Golgi-associated endoplasmic reticulum, but also diffusely in the cytoplasm of these cells (Fig. 8). The above biochemical and electron cytochemical findings of cytoplasmic tyrosinase activity

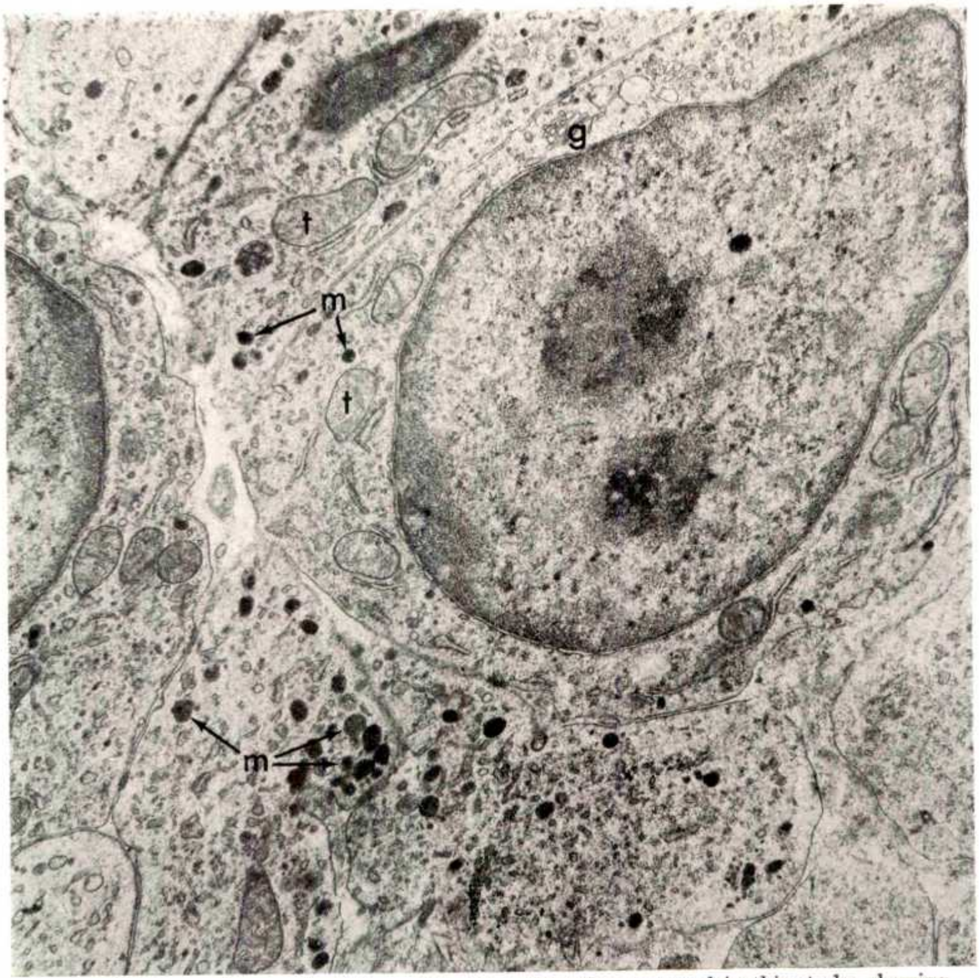

FIG. 7.—Electron micrograph of Fortner's melanotic melanoma used in this study, showing various subcellular compartments and their organization; m: premelanosome and melanosome; t: mitochondrion; g: Golgi apparatus. (× 18,000.)

can also be seen in other varieties such as Greene's (Mishima and Adams, 1969) and human melanomata (Mishima, 1966). McGuire (1970) has recently reported that the cytoplasmic soluble fraction obtained from epidermis of *Rana pipiens* acquires demonstrable tyrosinase activity after its treatment by chymotrypsin or trypsin. He has suggested the activation of protyrosinase as the mechanism for inducing demonstrable tyrosinase. In our case the soluble fraction of Fortner's melanotic melanomata contains already demonstrable tyrosinase activity without any such treatment. In addition, both amelanotic and melanotic melanomata exhibit highest tyrosinase inhibitor in the soluble fraction. The present findings may suggest that the cytoplasmic soluble tyrosinase in these melanoma cells is

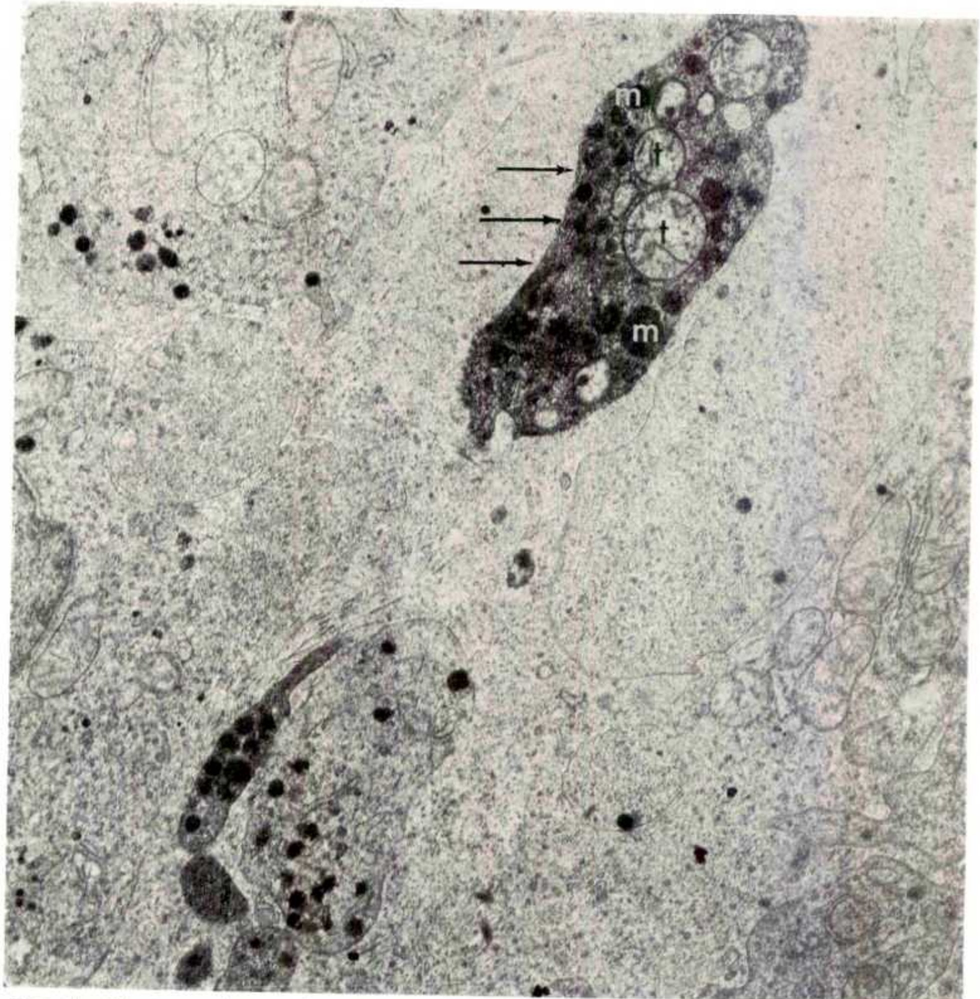

FIG. 8.—Electron micrograph of Fortner's melanotic melanoma after the dopa reaction, exhibiting induced melanization diffusely in the cytoplasm (←) and locally in the premelanosomes (m); t: mitochondrion. (×21,000.)

inhibited *in vivo* by tyrosinase inhibitor, representing at least 1 of the factors controlling melanogenesis.

The melanogenic defect in amelanotic malignant melanoma cannot always be attributed to an absence of tyrosinase or premelanosome synthesis, since both can be found in a great number of these cells. In 1966 we reported that characteristic differences in the distribution of tyrosinase among the large granule subfractions exist between amelanotic and melanotic melanomata. In contrast to melanotic melanoma, amelanotic melanoma large granule subfractions 3 and 5, which have been shown to consist primarily of mitochondria and premelanosomes, contain very little tyrosinase activity (Mishima *et al.*, 1971; Mishima and Cooper, 1966). Correspondingly, the absolute amount of tyrosinase inhibitor in amelanotic melanoma is greatest in subfractions 3 and 5 (Fig. 3), although it is present in all the subfractions in higher concentration than in melanotic melanoma. Our findings suggest that disturbed melanization occurring in premelanosomes of amelanotic melanoma *in vivo* is directly related to the higher concentration of tyrosinase inhibitor in these organelles.

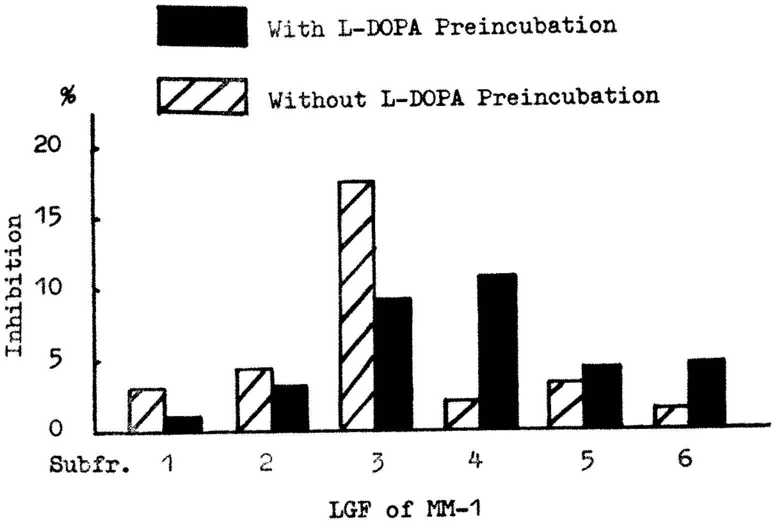

FIG. 9.—Intracellular distribution of the tyrosinase inhibitor among large granule subfractions (LGF) of Fortner's melanotic melanoma, with and without dopa preincubation, exhibiting a distinct shift of its activity from subfraction **3** to 4, 5 and 6.

In our earlier investigation of tyrosinase inhibitor we were confronted with its high concentration in large granule subfraction 3. It has been reported (Seiji *et al.*, 1963; Mishima, 1966) that the subfractionation technique employed here separates mitochondria into subfraction 3 and premelanosomes and melanosomes into subfractions 4, 5 and 6; yet the defect of melanization in amelanotic melanoma would seem to reside not in the mitochondria but in the premelanosomes. Subsequent assay of our subfractions for the mitochondrial enzyme succinoxidase reveals an almost exclusive concentration of the enzyme in large granule subfraction 3 (Table I, II). However, electron microscopy of this subfraction with and without dopa pre-incubation demonstrates a substantial number of premelanosomes in addition to the mitochondria (Fig. 4, 5, 6). Furthermore, tyrosinase inhibitor assay of dopa preincubated subfractions shows a distinct shift in the activity from 3 to 4, 5 and 6, indicating that the inhibitor seen in subfraction 3 is largely contained within the premelanosomes (Fig. 9). The inhibitor remaining in subfraction 3 after dopa incubation is a subject for speculation. It is possible that this residual activity is contained within the remaining population of premelanosomes we have found by electron microscopy (Fig. 6). This finding adds further support to the possibility that the defect of melanization in the premelanosomes of tyrosinase-containing amelanotic malignant melanoma may be attributed to the tyrosinase inhibitor concentrated in these specific organelles.

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ULTRASTRUCTURAL DIFFERENCES IN HUMAN MALIGNANT MELANOMATA

AN ELECTRON MICROSCOPICAL STUDY

HERBERT KLUG AND WOLFGANG GÜNTHER

Summary.—Thirty-two human malignant melanomata have been investigated with the electron microscope. On the basis of the fine structure of the melanomata and melanosomes we have distinguished 2 different tumour forms (Type A and Type B). The melanosomes of Type A appear cigar-shaped, whereas the premelanosomes have an internal helical structure exhibiting periodicity. Most of the mitochondria of this tumour type show an elongated form and numerous cristae. The nucleolus is usually very large and exhibits some vacuole-like structures which contain a fine granular substance. The nucleus is distinguished by the different extent of heteropyknotic chromatin and frequently has " nuclear bodies ". The tumour cells of the other melanoma form (Type B) contain only spherical melanosomes, whereas the premelanosomes show no internal fibrillar structure. The mitochondria mostly appear spherical as well; their matrix is slightly electron dense. There is no marked difference between the nuclei of these 2 melanoma forms. Their pathogenesis is discussed.

MALIGNANT melanomata have a wide range of malignant potential, and little is known concerning the relationship between their biological behaviour and their morphological structure (Lane *et al.*, 1958; Mishima, 1965; Gertler and Thormann, 1968; Hardmeier *et al.*, 1968; Cochran, 1969; Hardmeier, 1970). Furthermore, it has been known for many years that great structural variety is a most important feature of human malignant melanomata. The variations in biological behaviour are not clearly associated with the degree of pigmentation, or of mitotic activity, (Lewis and Kiryabwire, 1968; Günther, 1970), although many authors have assumed that amelanotic tumours are dedifferentiated and therefore more malignant than melanotic ones (Albertini, 1952; Kretzschmar and Schwarzer, 1960; Lever, 1962). Moreover, some authors have affirmed that melanomata composed of fusiform cells have a better prognosis than those composed of polymorphous melanocytes (Albertini, 1955; Greither and Tritsch, 1957). This assumption has been denied by other authors (Ackerman, 1949; Catlin, 1954; Gartmann, 1958).

Recently, Mishima (1967) found that the melanomata derived from a junction naevus differ from those developing from circumscribed precancerous melanosis of Dubreuilh. Furthermore, this author has established that melanomata arising from precancerous melanosis differ from those originating from junction naevus in their slower growth, later metastasis and greater radiosensitivity. Therefore Mishima has distinguished 2 forms of malignant melanoma: naevocytic and melanocytic. However, these observations were not confirmed by Klingmüller *et al.* (1970).

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In a previous study on the ultrastructure of junction naevus, precancerous melanosis and malignant melanoma, it was shown that there may be different forms of melanoma (Klug and Thormann, 1971). It was therefore the purpose of the present investigation to examine these tumours in detail. We report electron microscopical examinations of the fine structure of malignant melanomata obtained from different sources.

MATERIAL AND METHODS

We have used 32 human cutaneous malignant melanomata, most of them primary tumours. Specimens from the melanomata were obtained after excision, and small fragments, about 1 mm^3, were subjected to fixation for 2 hours at room temperature in a 1% buffered (pH 7·3) OsO_4-solution. Fixation was followed by washing in isotonic solution, dehydration in graded alcohols and subsequently in acetone, and, finally, embedding in Vestopal W. Thin sections were cut on an LKB-ultratome, stained with uranyl acetate and lead citrate, and examined with the electron microscope (60–80 kV).

RESULTS

Fig. 1 is a low-power micrograph of part of a melanoma cell. Disregarding the nucleolus, which has not been cut, this tumour cell may be considered as characteristic of the most malignant melanomata which have been investigated here (Type A). It is of interest that the numerous mitochondria in these melanoma cells are mostly arranged in the perinuclear cytoplasm. Sometimes these organelles are so abundant that they almost entirely fill the cytoplasm. Usually, the mitochondria exhibit a longish form and have a very well-developed internal structure (cristae). The cristae are oriented at right angles to the long axis. Only the peripheral cytoplasm has a sparse mitochondrial population. This typical shape and fine structure of the mitochondria has not been observed in all the tumour cells of the same melanoma, as is clearly to be seen in Fig. 2. This micrograph shows mitochondria from another tumour cell of the same melanoma, but at a higher magnification. All mitochondria appear more or less spherical and exhibit a less electron-dense matrix, as well as only a few cristae. Intramitochondrial granules are rarely present. We have seldom observed such tumour cells in this type of melanoma. Furthermore, these melanoma cells contain numerous melanosomes in various stages of development. They are mostly aggregated in the peripheral cytoplasm, although a few appear between the mitochondria (Fig. 1). The melanization of the premelanosomes is incomplete, and the inner structure can be discerned. Fig 3 shows a high-power micrograph of some melanosomes and premelanosomes which are typical for this melanoma form (Type A). A fibrillar inner structure can be seen along the long axis, in which a striated or helical pattern is present.

The difference between the premelanosomes seems to be a result of the variable degree of deposition of melanin on this basic framework; but, in general, there was no specific topographical relationship between premelanosomes and the Golgi-apparatus, which appears to be poorly developed in these melanoma cells. The Golgi-apparatus is usually located supranuclearly and consists of a few smooth-surfaced lamellae and small, flattened, smooth-walled vesicles. The individual melanin granules are ellipsoidal bodies and are of varying size, but on average they measure 800 × 300 nm.

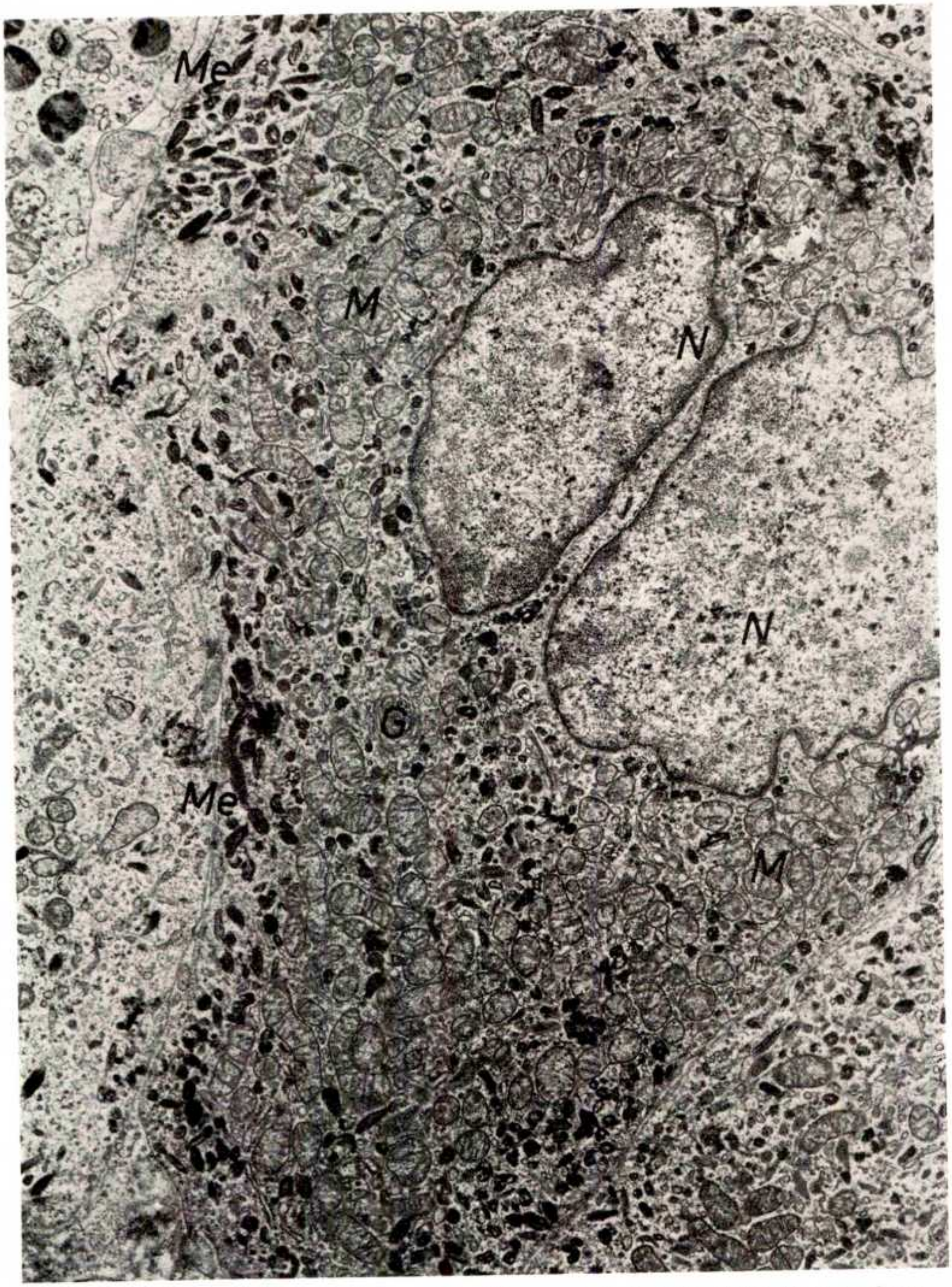

FIG. 1.—Malignant melanoma cell (Type A) with many mitochondria (M) in the cytoplasm and numerous melanosomes, mostly peripherally aggregated. G = Golgi-apparatus, Me = melanosomes, N = fragments of the nucleus (× 18,000).

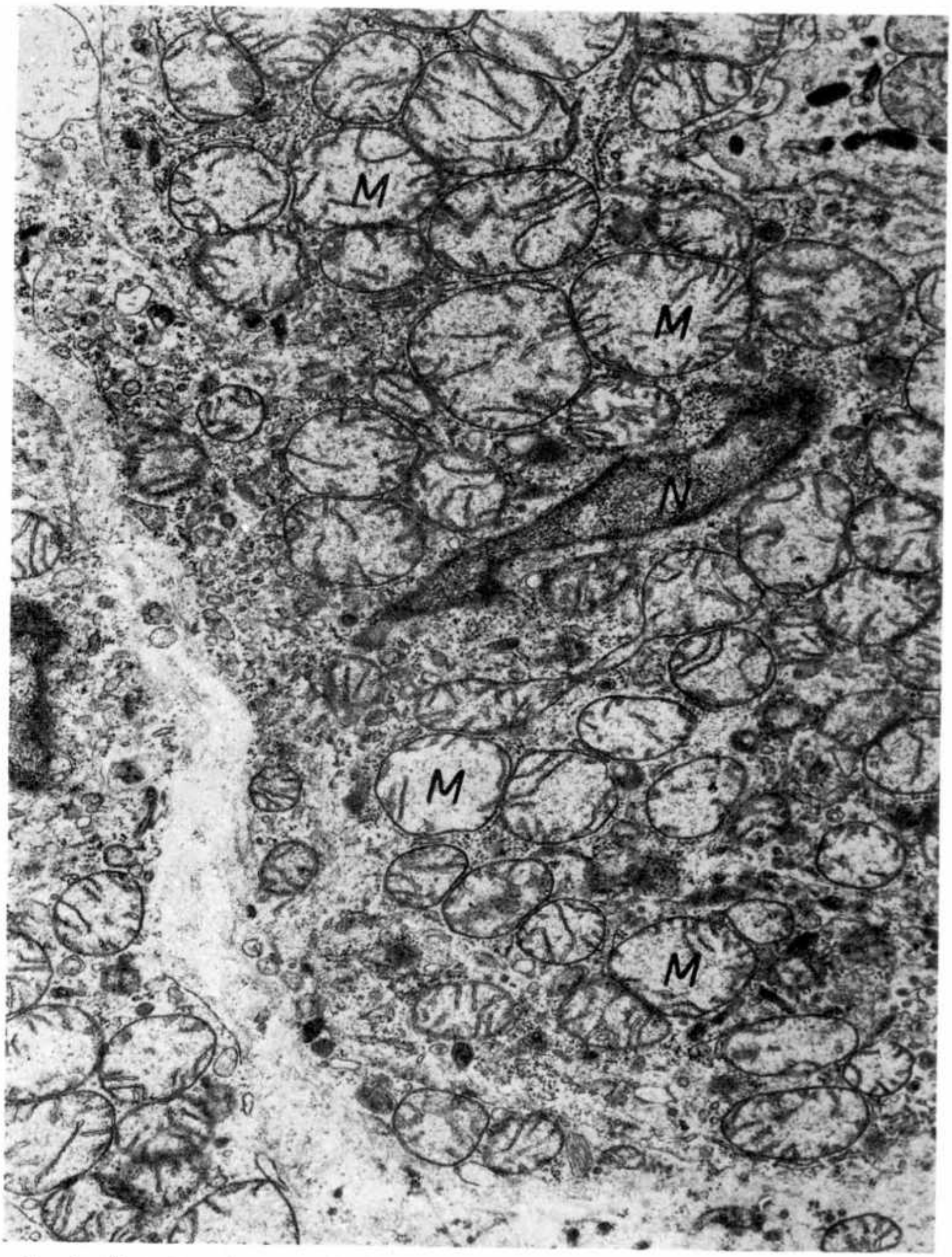

FIG. 2.—Part of a melanoma cell (Type A) with numerous mitochondria (M). Among them are many ribosomes. N = nucleus (× 30,000).

Besides melanosomes, premelanosomes and mitochondria, the cytoplasm contains numerous ribosomes, polysomes and sparse smooth-surfaced endoplasmic reticulum, but no lysosomes or phagosomes. The nuclei usually show several indentations and deep invaginations of the nuclear envelope (Fig. 4). The chromatin structures are irregularly arranged in the space of the nuclei and consist of eupyknotic and heteropycnotic parts. Frequently, " nuclear bodies " can be observed in these melanoma cells, which are different in structure and size.

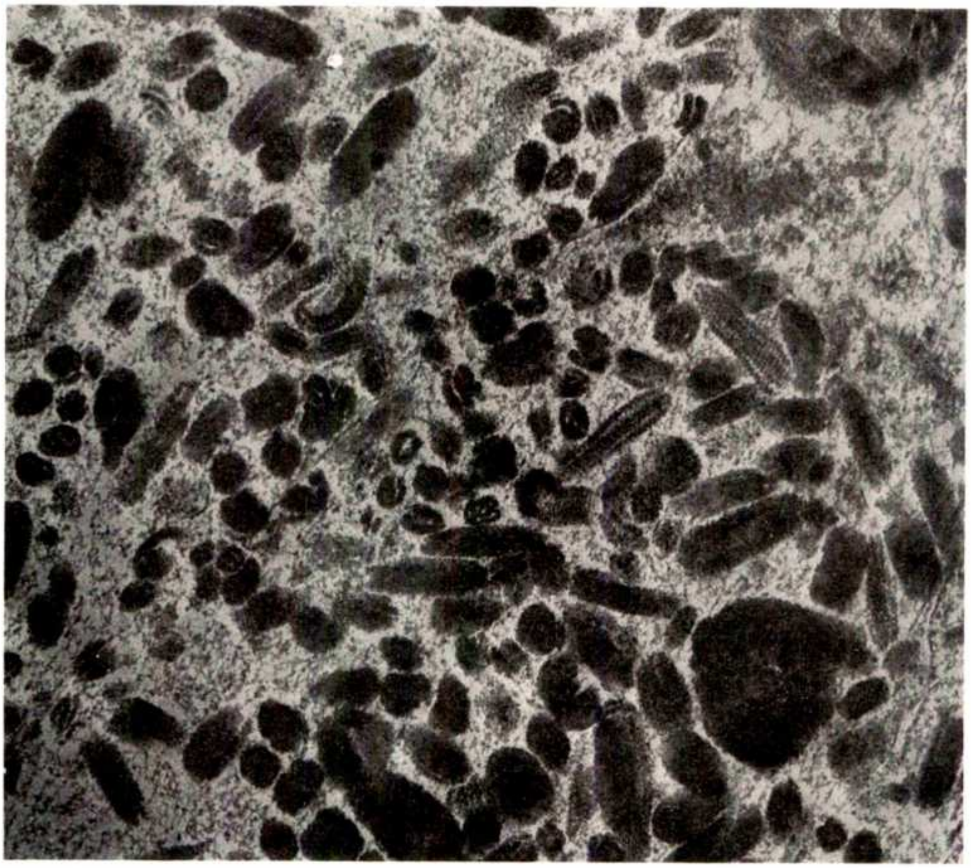

FIG. 3.—Melanosomes and premelanosomes of a tumour cell (Type A) (× 90,000).

It is remarkable that the nuclei contain 1 or 2 very large nucleoli. The latter appear as electron-dense, rounded bodies, mainly eccentrically placed in the nucleus. No membrane separates them from the rest of the karyoplasm. The ultrastructure of these organelles shows 2 regions, 1 apparently more dense than the other (Fig. 4). The denser material is composed of granules or fibrils with a diameter of approximately 75 nm. The less dense material is of the same size. A most interesting feature of the nucleoli, in addition to their considerable enlargement, is the presence of various large, less electron-dense formations which appear rather like vacuole-like structures. These formations contain fine gran-

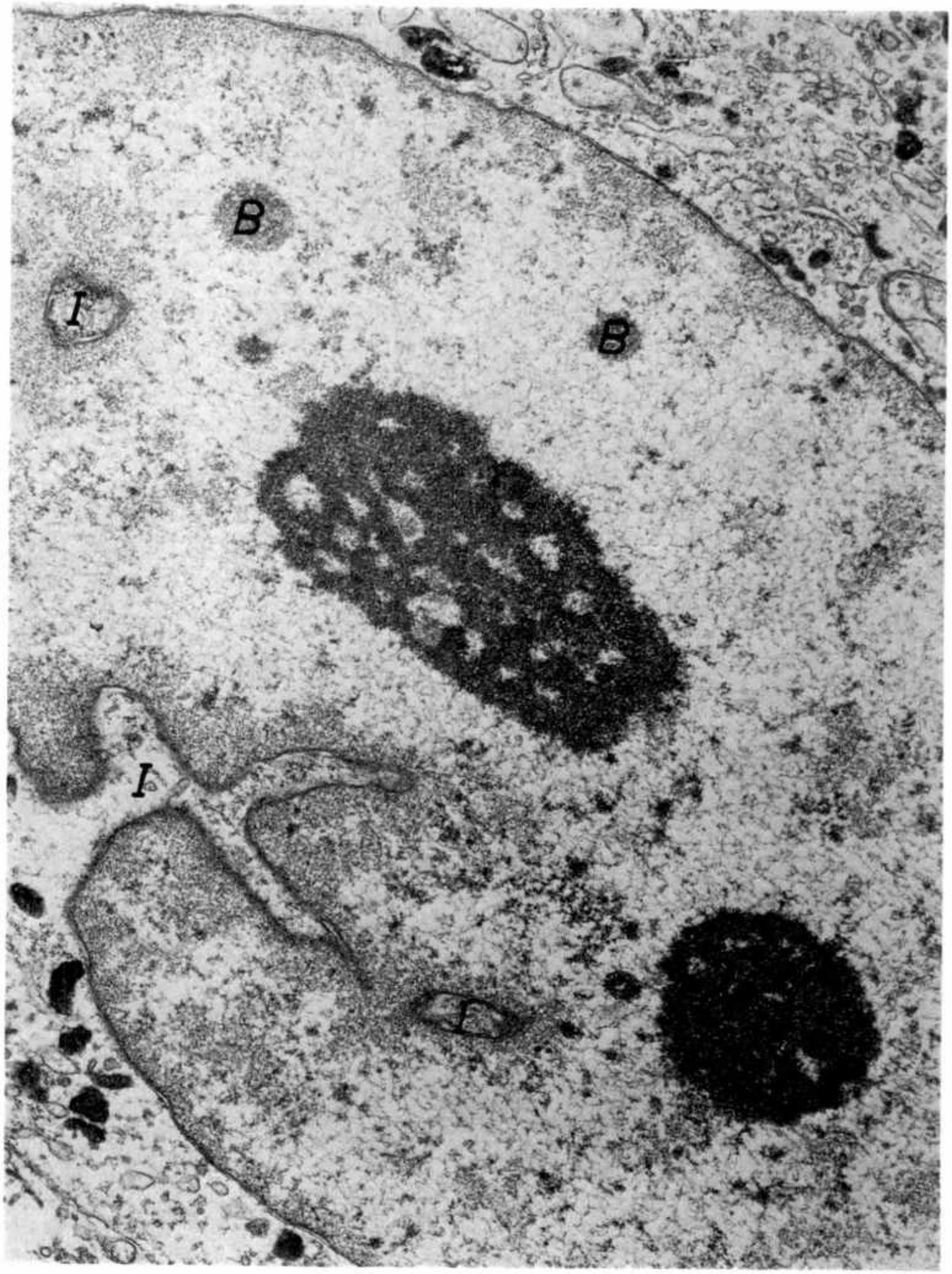

FIG. 4.—Melanoma cell nucleus (Type A) with 2 nucleoli and deep invaginations (I). B = nuclear bodies (× 30,000).

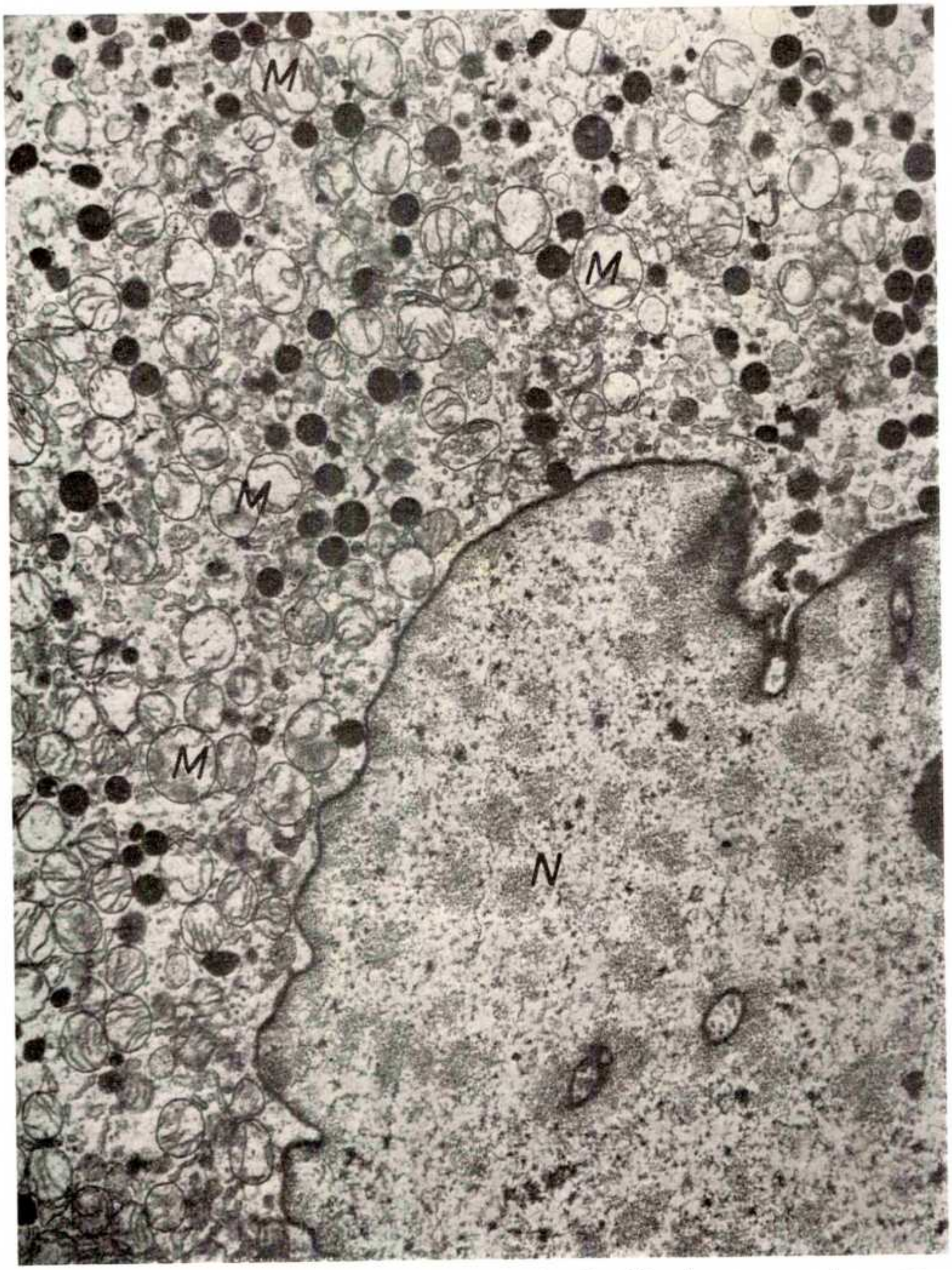

FIG. 5.—Melanoma cell (Type B) with many mitochondria (M) and numerous melanosomes which are regularly distributed in the cytoplasm. N = nucleus (× 18,000).

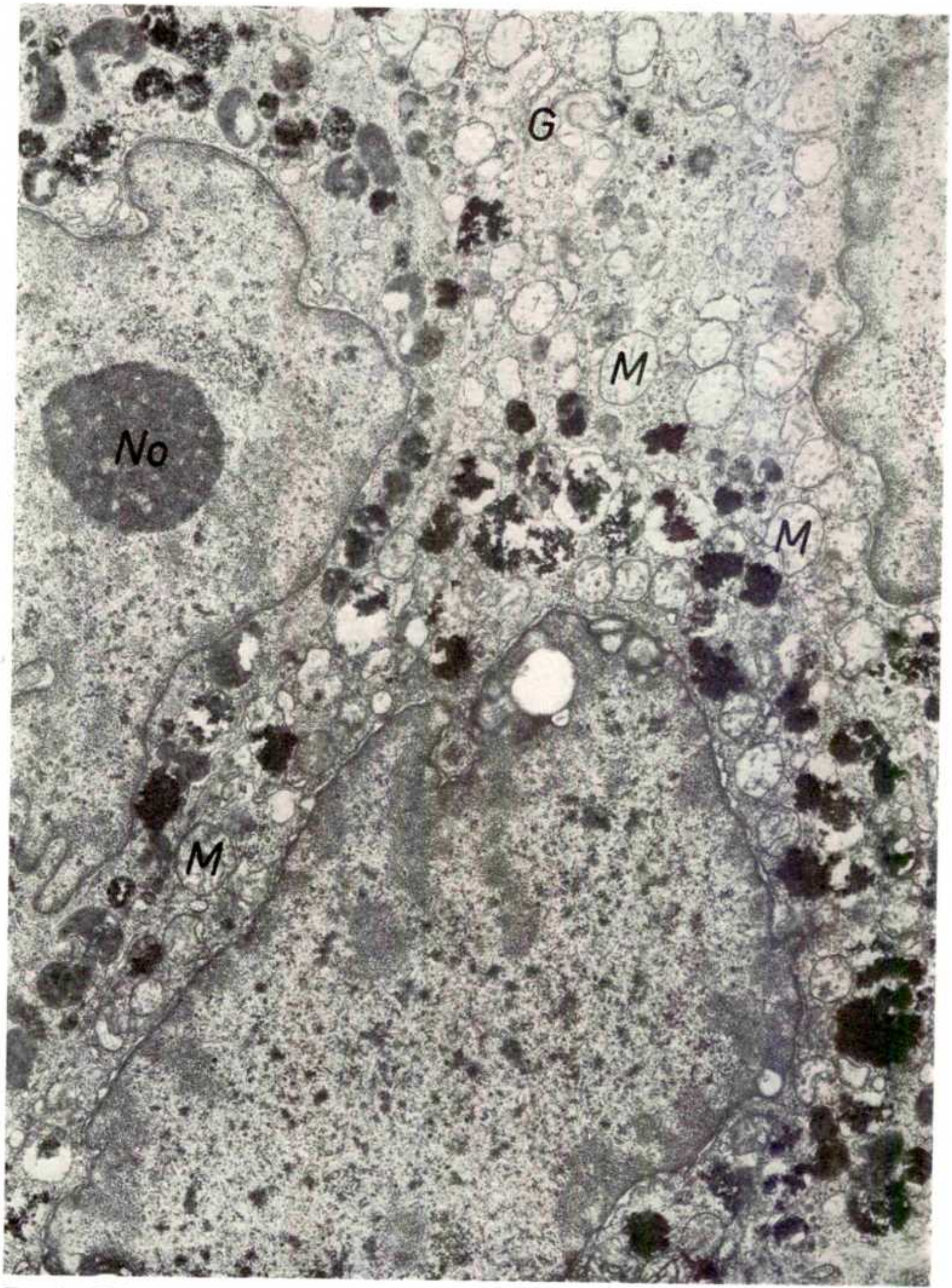

FIG. 6.—Melanoma cells (Type B) with numerous melanin-ferrous inclusions of the cytoplasm G = Golgi-apparatus, M = mitochondria, No = nucleolus (× 18,000).

ules which are embedded in an amorphous matrix. Sometimes the nucleoli are closely attached to chromatin structures. In the course of our investigations we have observed some melanomata (8), which we have called Type B, which show a striking ultrastructural difference from the other melanoma form (Type A). A survey micrograph of these B-type melanoma cells is provided (Fig. 5). It is noteworthy that the spherical, very electron-dense melanin granules are distributed throughout the whole cytoplasm. The diameters of these melanosomes range from 250 to 500 nm. Between the dense granules are some which clearly exhibit a granular substructure. In this respect these melanoma cells differ markedly from the others. The cytoplasm of these melanoma cells also contains, similarly, numerous mitochondria. However, most of them exhibit a spherical form, only a few cristae, and a less electron-dense matrix. It is noticeable that the free ribosomes are less numerous than in the Type A melanoma cells. No consistent differences were noted in the amount of endoplasmic reticulum or Golgi-apparatus.

TABLE I.—*The Most Important Morphological Features for Comparing the Classification of Mishima with Our Own*

	Naevocytoma	Melanocytoma	Type A	Type B
Melanosomes	Cigar-shaped 850 × 350 nm	Ellipsoid 500 × 450 nm	Cigar-shaped 800 × 300 nm	Spherical 250 to 500 nm
Premelanosomes	Helical structure	Granular structure?	Helical structure	Granular structure
Mitochondria	Not described	—	Longish numerous cristae	Spherical few cristae

The nuclei of Type B melanoma cells are considerably misshapen. Usually they have numerous indentations and are also deeply incised at one or more places (Fig. 6). Likewise, 1 or 2 large nucleoli appear within the karyoplasm. In comparison with the nucleoli of Type A cells, no striking variation was found in this study, although the organelles of this tumour type may be generally a little smaller.

It will be of interest that the tumour cells of 2 melanomata of this group exhibit a slightly different ultrastructure (Fig. 6). The cytoplasm of these cells also contains numerous melaniferous granules of varying size, which appear to be partly vacuolar. These manifold inclusions do not show a distinct inner structure. To some extent they resemble morphologically the phagolysosomes of the melanophages, but their mitochondria and other components of the cytoplasm agree with those of the other tumour cells of this type of melanoma.

DISCUSSION

The present study of human malignant melanomata has revealed that there are distinct ultrastructural differences between the individual tumours which we have divided primarily into 2 groups. These morphological differences are found mainly in the submicroscopical structure of the premelanosomes, melanosomes and mitochondria. In this paper the term " melanosome " is synonymous with the term " melanin granule " because it is very difficult to determine precisely the onset of melanin synthesis and therefore to distinguish between " premelanosome " and " melanosome ".

For the present, these 2 groups are named the A- and B-forms (A- and B-types). It is noteworthy that within a melanoma of the B-form we have not found tumour cells with typical melanosomes of the A-form or vice versa. Sometimes the internal structure of the premelanosome appears as concentric, spiral strands in their presumed cross-sections. The premelanosomes of the A-form display, more or less distinctly, in all melanin-containing melanomata, fibrillary structures which are arranged along the long axis. At higher magnification, these fibrils show a helical respectively coiled structure. Such premelanosomes have also been found in normal melanocytes of the epidermis and of the hair bulb (Birbeck, 1963, 1962; Drochmans, 1966, 1963). Premelanosomes of this structure have been observed in intra-epidermal cells of the benign juvenile melanoma (Schreiner and Wolff, 1970) and in cells of naevus cell naevus (Gottlieb *et al.*, 1965). These typical premelanosomes were also seen in pigmented cells of the mouse melanoma (Stäubli and Loustalot, 1962) and golden hamster melanomata (Klug and Thormann, 1969). It is noteworthy that similar premelanosomes have been found in the cells of precancerous melanosis (Mishima, 1967).

Finally, the malignant melanomata of the human skin contain such melanosomes and premelanosomes (Wellings and Siegel, 1960, 1963; Zelickson, 1962) the structure of which may thus be the most frequent form of the benign and malignant pigment cells. These results are not completely in accord with the findings of Mishima (1967), who has described cigar-shaped melanosomes in 1 melanoma which had developed from a junction naevus. These melanosomes are indistinct in outline and show a partially disintegrated structure, measuring 350 × 850 nm. We have not found such melanosomes in this type of melanoma. Furthermore, ellipsoid melanosomes, averaging up to 250–500 nm, have been described in 1 melanoma originating from a precancerous melanosis (Mishima, 1966). This average size roughly corresponds to the diameter of the B-form spherical melanosome. In some places the less electron-dense melanin granules exhibit fine granular structures. Neither vertical nor longitudinal fibrillar structures have been observed.

The melanosomes of amphibian melanophores (Wise, 1969) and fish have similar forms (Fujii, 1966; Fujii and Fujii, 1966). Jakubowicz *et al.* (1970) have observed granular structures more frequently than fibrillar or granular structures only. We cannot agree with the opinion of the named authors that the granular structures may be degenerated, since we have not seen premelanosomes with fibrillar structure in tumour cells from corresponding melanomata. Furthermore, no connection could be traced between such granules and degenerative changes in other cell structures. Similar spherical melanosomes and premelanosomes (though the latter seem a little coarse-granulated) have been observed is *in vitro* experiments in melanoma cells (Stank *et al.*, 1969) as well as partially in melanocytes of freckled human epidermis (Breathnach and Wyllie, 1964).

From the morphological point of view, the spherical melaniferous granules which have been observed in this study bear a slight resemblance both to the lysosomes and to the phagolysosomes. This applies especially to those structures of the cytoplasm which we have observed in all tumour cells of 2 malignant melanomata (Fig. 6). Furthermore, it appears that these melanin-containing inclusions do indeed differ from the phagolysosomes of the melanophages (Klug and Thormann, 1969) and do not present compound-melanosomes such as are found in melanocytes of normal epidermis (Hori *et al.*, 1968). It may be concluded that these cells are malignant melanoma cells and not melanophages, on the one hand from their ultrastructure, and, on the other, from the established fact that the 2 melanomata show only such cells in all fields examined. Moreover, it is noticeable that the clinical course and features were essentially similar in both cases, and metastases were found at hospitalization. At present we can say nothing about the prognosis, and we have no evidence that the clinical course for these 2 cases is different from the others.

Electron microscopic investigations on cell cultures from 2 human malignant melanomata (Maul and Romsdahl, 1970) support the suggestion that such melanin-containing structures, as discussed above, may be partly lysosomes. In addition, these investigations have shown that the melanomata examined represent 2 obviously morphologically different tumour lines, 1 of them corresponding to the described Type A unequivocally, whereas the other, which has no premelanosomes with helical structure, approximates to Type B. Moreover, in these melanin-containing inclusions established acid phosphatase activity seems to point to the lysosomal nature of these structures. Acid phosphatase activity was present in aggregate forms of melanosomes within melanophages in precancerous melanosis (Mishima, 1966).

As we could not detect any lysosomal-like structures within the Type A melanoma cells, later investigations may show the possibility of relations to the clinical course, but at present no definite correlation can be found between these cell structures, these tumour types, and the clinical course and features respectively. The single, electron-dense melanin complexes which have been observed in these melanoma forms may be secondary lysosomes. It may be that these variations are due to the following secondary factors as well as to unknown intrinsic factors (degradative processes of melanosomes, distinction of melanophages) (Mishima, 1966).

As already noted, Mishima (1965) has reported on 2 different melanomata with various melanosomes which have been termed " naevocytic " and " melanocytic " melanoma. A similar observation was made by Cesarini *et al.* (1969), whereas Klingmüller (1970) could not find such differences.

In our present investigation there is general evidence of morphologically different melanomata. Both the melanosomes and the mitochondrial structures refer to 2 different tumour forms, although the ultrastructure of these organelles may certainly be affected by their function and age. Indeed, it may be of interest that the cristae of Type B melanoma cells have almost disappeared in most cells and the mitochondrial matrix shows a diminished electron density. Therefore careful consideration of the fine structure of these melanoma cells leads to the conclusion that there are differences between the 2 tumour types.

The fine structure of the nuclei shows no striking difference between the 2 types of melanoma. Of course, it appears that the nucleoli of Type A are considerably larger than those of Type B and that the ultrastructure of the latter is more normal than the other. It is therefore suggested that this greater size may be essential to the clinical course. The importance of the enlarged nucleoli in tumour cells has been discussed in a previous paper (Klug and Thormann, 1971). In addition, in comparison with Type B, the nucleoli of Type A show an increased number of vacuole-like structures, whereas this organelle in the other melanomata exhibits more clearly differentiated nucleonemata.

Furthermore, it will be of interest that there are some indications for assuming that the melanomata of the B-form (8 cases) correspond to the " melanocytic " melanoma described by Mishima (1966). Indeed, this author's results have been obtained from 1 melanoma developing from precancerous melanosis. Our assumption, however, is supported by the fact that the melanocytes of this disorder contain mostly spherical melanosomes and spherical mitochondria (Klug and Thormann, 1971), although in general the melanosomes appear highly polymorphous and are sometimes cigar-shaped, too. Histologically all melanomata of this type are polymorphous or globular and mostly disorderly. Therefore it seems impossible to decide, either histologically or ultrastructurally, precisely whether a melanoma is developing from junction naevi or from melanosis. In addition, it may be of interest that none of the tumours investigated has been found on the face, whereas it has been established that precancerous melanosis is located mainly on the face (Gertler and Thormann, 1968).

Taken together, however, the findings obtained from the present study show that the fine structures of the melanomata differ. Yet it seems exceedingly difficult precisely to determine their origin.

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COMBINED CYTOSTATIC AND SURGICAL TREATMENT OF EXTENSIVE BASAL CELL EPITHELIOMATA

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Summary.—Two essentially similar cases of extensive basal cell epitheliomata of the face are reported, which had been treated with x-rays without a definite cure and were treated by us with 5-fluorouracil ointment until the epithelioma was totally destroyed. This was followed by free transplantation of a split skin flap with primary healing and an excellent cosmetic result. The method offers an alternative treatment for extensive basal-cell epitheliomata which are not cured by radiotherapy and operative measures.

CHEMICAL and cytostatic treatment alone or in combination with surgical intervention for basal and squamous cell epithelioma are used increasingly often, especially for recurrences after x-ray treatment. According to all reports, any such therapy has its limitations, depending on the type of tumour, its localization, its extent and the previous treatment given, limitations which necessitate a change of treatment in certain cases. None of these much discussed forms of therapy is a recent introduction. There is, for example, a report by Heep (1949) on the treatment of superficial epitheliomata with colchicin ointment. Schreus (1951) recommended zinc chloride treatment of epitheliomata, and Belisario (1954) introduced a new form of treatment with chemo-surgery. Besides colchicin, used by Heep (1949), Belisario used Thiocolceran (1954), aminopterin (1961) and Triaziquon (1965). Since the discovery of 5-fluorouracil and its cytostatic effect by Heidelberger and colleagues in 1947, this substance has been increasingly used as topical therapy for malignant skin tumours. Favourable results have been confirmed by Dillaha *et al.* (1963, 1965), Schirren and Gruber (1964), Honeycutt (1968) and Goddard (1968).

Operative measures in combined chemo-surgical therapy are normally confined to the initial cautery destruction of the tumour to make the tissues more accessible to local cytostatic treatment.

The reverse order, i.e. initial tumour destruction by a locally applied cytostatic with subsequent surgical repair of the defect produced, has to our knowledge not yet been tried. It is appropriate in such situations as are described here, i.e. in an extensive rodent ulcer. Two cases, unusual because identical, are reported where the ulcer at first spread considerably after local treatment with 5-fluorouracil. This spread was due to the intradermal extent of the tumour, not visible clinically and recalling the traditional iceberg. Cytostatic action naturally destroyed the

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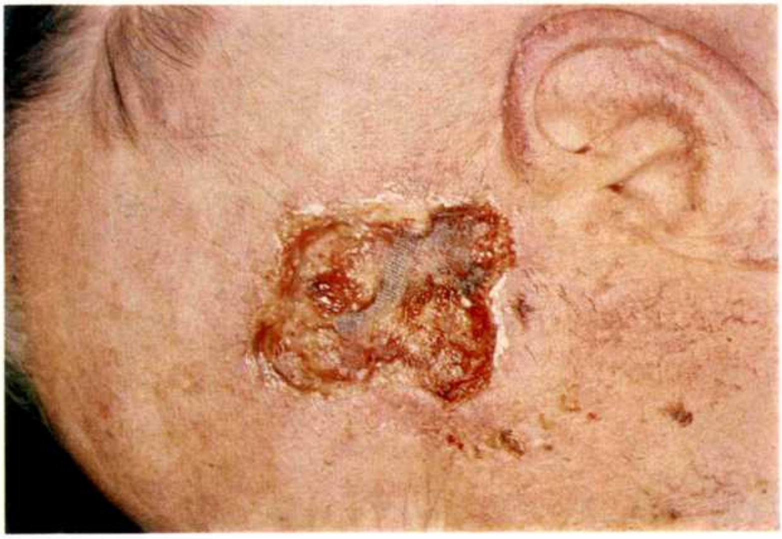

FIG. 2.—The same, 6 weeks after local application of 5% 5-fluorouracil ointment.

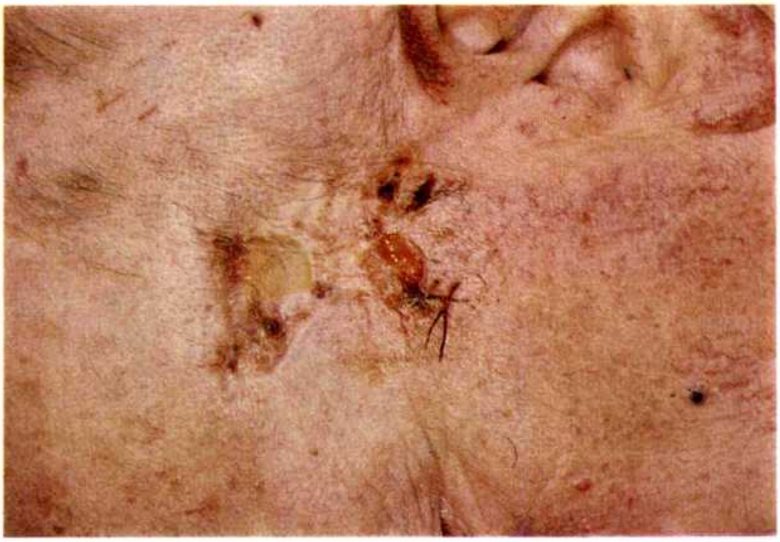

FIG. 1.—Ulcerating recurrent basal cell epithelioma before cytostatic treatment (Case 1).

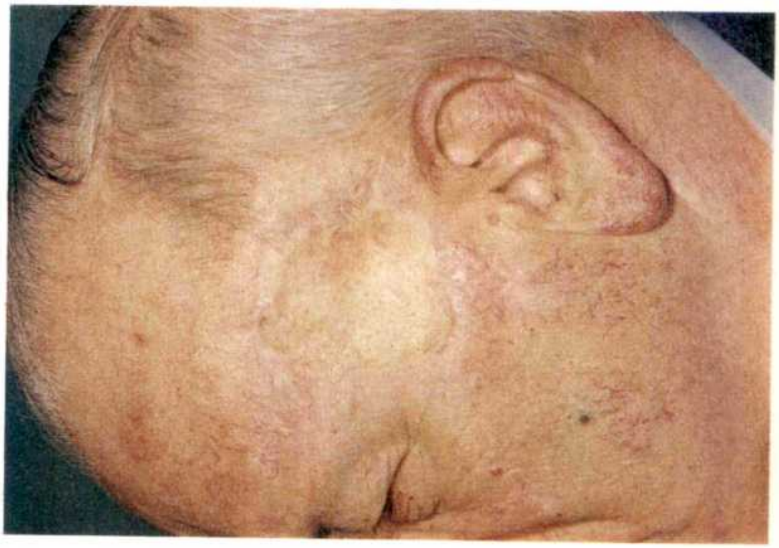

FIG. 4.—Final result.

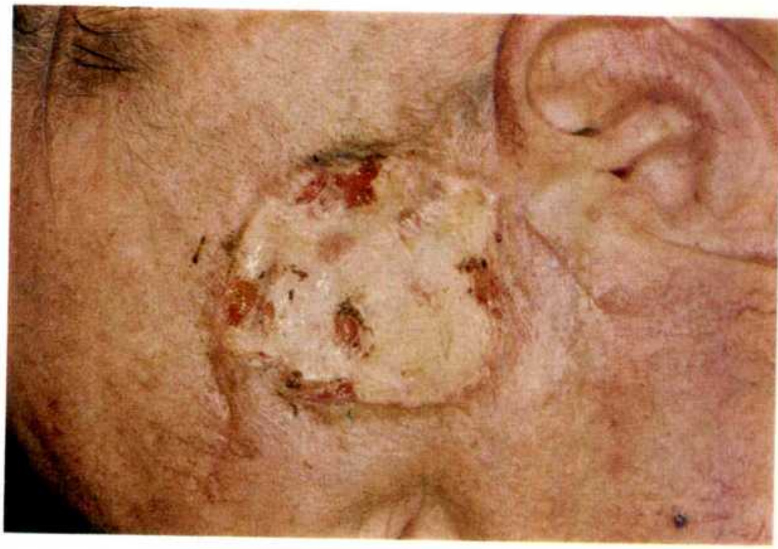

FIG. 3.—After grafting.

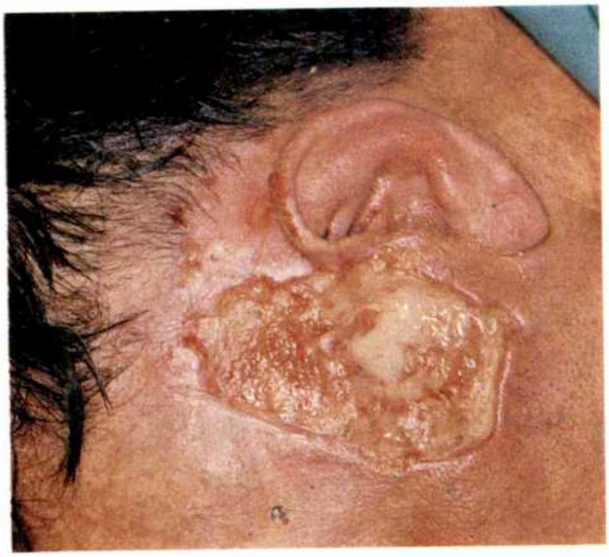

FIG. 6.—Extension of defect under 5-fluorouracil treatment.

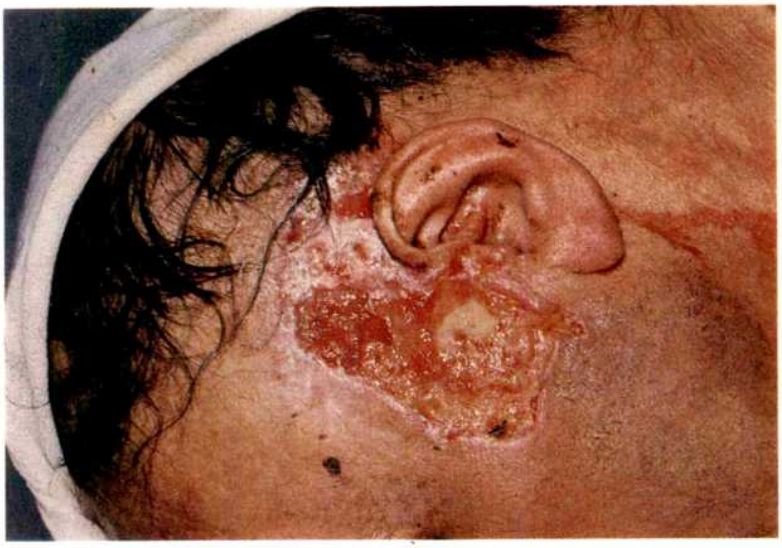

FIG. 5.—Rodent ulcer before treatment (Case 2).

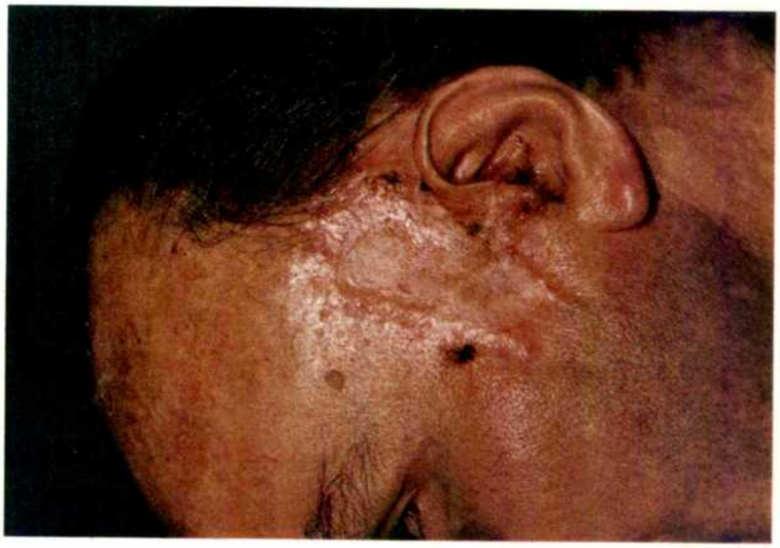

FIG. 7.—Final result after grafting.

tumour, with the formation of a deep and wide defect. It was possible to cover this defect by a free graft, which ensured a satisfactory cosmetic result.

CASE REPORTS

Case 1

The patient was a man aged 69. Nine years ago he noticed the appearance of a nodule about 3 mm in diameter in front of the left ear. It was diagnosed as a basal cell epithelioma and treated by his doctor with diathermy followed by radiotherapy. Seven years later a probable recurrence was noticed in the scar. Repeated radiotherapy of unknown dosage achieved temporary healing, but later another recurrence developed which steadily increased in size during the 9 months immediately preceding admission to hospital.

Skin condition on April 20th, 1970 (Fig. 1).—In front of the left ear, extending towards the temple, there was a typical basal cell epithelioma about 3·5–4 cm in diameter, forming in 2 places ulcers about 1·5 cm in diameter, crowned by a greasy crust. The surrounding skin showed x-ray atrophy and numerous teleangiectases. Near the large defects there were isolated, small, recent ulcers. Histological examination confirmed the diagnosis of ulcerating basal cell epithelioma.

Therapy and course.—For 6 weeks we carried out local cytostatic treatment with 5% fluorouracil ointment*. Application was usually made each day with polythene occlusive dressing, and protection of the surrounding area was effected with a fat-free powder suspension. Definite extension of the 2 ulcers to a total size of 4·8 × 5·7 cm and a depth of about 0·3 cm followed (Fig. 2). In 6 weeks of treatment the destruction of the undermining epithelioma had produced a defect free from tumour. In order to confirm the result of our treatment, deep biopsies were made on both sides and above and below the defect in the surrounding, clinically unaffected skin. Histologically, no epithelioma tissue was found either in these sites or in the base, which penetrated to the fascia and in places exposed the skull. This allowed the conclusion that the epithelioma had been completely destroyed by the cytostatic treatment. The defect was narrowed by 2 tension-relieving incisions, under general anaesthesia, in the preauricular frontal area (Fig. 3). In a second operation about 10 days later we covered the defect with a split skin flap from the left thigh. The graft was fixed with catgut sutures, and some openings were made in its centre as an outlet for secretions. The graft healed completely, despite the x-ray atrophy of the area (Fig. 4).

Case 2

The patient was a man aged 60. A basal cell epithelioma had been present on the left temple for 6 years. During this period it was given 3 or 4 doses of x-ray, each of 4500 R, by the dermatologist in charge. This was said to have caused some shrinkage of the tumour, but no complete healing. Three months before his admission to hospital renewed tumour growth was observed.

Skin condition on June 30th, 1970 (Fig. 5).—A basal cell epithelioma measuring 7·3 × 4·3 cm, was present in the left preauricular region. In an area some 2 cm in diameter the jugular bone was covered only by necrotic periosteum. The edge of the ulcer was sharply elevated. Towards the healthy skin, especially at the lower pole of the defect, small thickened nodules could be felt. The ulcer was situated in the scar of a burn which had been present since boyhood. Histological examination of the edge of the tumour and ulcer confirmed the diagnosis of rodent ulcer.

Therapy and course.—Five per cent 5-fluorouracil ointment was applied with polythene occlusive dressings, with a view to destroying the epithelioma selectively and then covering the defect by operation. The therapeutic response was checked repeatedly by histology. At the end of the tenth week of treatment with 5-fluorouracil ointment the defect had increased to 7·2 × 4·8 cm (Fig. 6). Whereas during the first half of the period of treatment epithelioma tissue persisted at the lower pole of the defect, no tumour growth was demonstrable histologically by the end of the tenth week. After cleaning out the ulcer under general anaesthesia, a split skin flap was transplanted from the left thigh and fixed with catgut sutures. In this case, too, the graft healed without complications in the area of x-ray atrophy which had been further damaged by burns in childhood (Fig. 7).

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* Manufactured by Hoffmann-La Roche AG, Grenzach, Baden.

DISCUSSION

Occasional difficulties arise in the use of the classical forms of treatment, x-rays or operation, for basal cell epithelioma. Typical causes of failure are recurrences after radiotherapy or electro-cautery destruction, marginal recurrences, difficult sites and the large extent of the tumour. Whereas alternative therapeutic measures are available for all but the last situation, in the form of local chemical or cytostatic treatment, extensive or undermining rodent ulcer has always been a therapeutic problem. Neither radiotherapy nor plastic surgery alone was indicated in the cases described above. Systemic cytostatic therapy could not be carried out because of concurrent internal disorders, leaving only the therapeutic trial of consecutive local cytostatic agents and plastic surgery. In both cases, incidentally almost identical, the treatment produced total destruction of the epithelioma, as proved histologically by biopsy of the healthy margins and the ulcer itself. Operative debridement of the defect made possible the graft, which produced a thoroughly satisfactory cosmetic result. The use of the described variant of chemo-surgery is, however, indicated only for extensive rodent ulcer or for basal cell epithelioma growing intraepidermally.

This paper is dedicated to John C. Belisario, Sydney.

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Case Reports

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EXTRACTION OF PEMPHIGUS ANTIBODIES FROM A LYMPHOID NEOPLASM AND ITS POSSIBLE RELATIONSHIP TO PEMPHIGUS VULGARIS

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Summary.—A patient is described who had a malignant lymphoid neoplasm and later developed pemphigus vulgaris. Tumour homogenate from the neoplasm contained a high titre of pemphigus antibodies.

Since Beutner and Jordon (1964) demonstrated the presence of auto-antibodies to stratified squamous epithelium in serum from pemphigus vulgaris, various other reports have been published (Beutner *et al.*, 1965a, 1965b, 1968a, 1968b; Wright *et al.*, 1966; Waldorf *et al.*, 1966). It is now well accepted that immunofluorescent tests are invaluable for the diagnosis of pemphigus.

Reports of the association of pemphigus with systemic lupus erythematosus (Kough and Barnes, 1964; Chorzelski *et al.*, 1968; Whittingham and McKay, 1971) and myasthenia gravis (Strauss *et al.*, 1965; Beutner *et al.*, 1968c; Saikia *et al.*, to be published) are available. There is no report in the literature, to my knowledge, on the association of pemphigus with a neoplastic process which was proved directly responsible for the production of the pemphigus antibodies.

In the present case it is suggested that the pemphigus antibodies were produced by a malignant lymphoid neoplasm.

CASE HISTORY

The patient, a man aged 36, was admitted to the Western Infirmary, Glasgow in September 1970, with acute mouth ulcers and swelling of his legs.

The history began in the first week of August 1970 when he developed a low backache and swelling of his legs. He was admitted to the Royal Victoria Infirmary, Newcastle-upon-Tyne at that time, and investigations revealed that he had a para-aortic abdominal tumour causing partial vena caval obstruction. An enlarged axillary lymph node was biopsied and showed the histological features of giant follicular lymphoma (Brill–Symmers).

Within 2 days of his admission to our hospital, the patient exhibited the classical clinical picture of pemphigus vulgaris. This was confirmed histologically and with immunofluorescent tests.

Treatment was started with 60 mg of systemic prednisolone, without any improvement. The dose of steroid was increased to 280 mg per day, but, in spite of this high dose, the patient's skin and general condition deteriorated, with extensive skin and buccal ulceration and toxaemia.

At this stage the possibility that the tumour might be producing pemphigus antibodies was considered. As surgical or radiotherapeutic treatment of the tumour was

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thought impracticable, chlorambucil 10 mg per day was given by mouth in addition to 280 mg of steroid. After 2 weeks of this therapy there was further deterioration of the patient's condition. The dose of chlorambucil was then reduced to 5 mg per day, and methotrexate 5 mg per day was started. Methotrexate is believed by some to be more specific therapy for pemphigus. No response was seen with this regime and the patient's condition gradually deteriorated until he died on 12.11.70.

Apart from the presence of the pemphigus antiepithelial antibodies in his serum, the only other abnormal routine laboratory finding was an elevated E.S.R. of 43–47 mm in the first hour (Westergren).

MATERIAL AND METHODS

Antihuman IgG, IgA and IgM produced in animals* was conjugated with F.I.T.C. (Wick and Beutner, 1970).

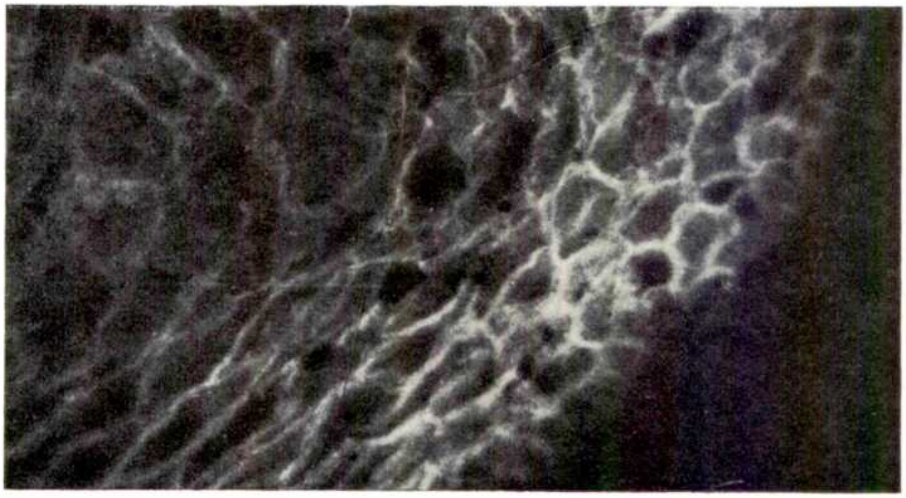

FIG. 1.—Indirect immunofluorescence test with patient's tumour homogenate extract on guinea-pig lip. Intercellular immunofluorescence with anti-IgG conjugate. Positive to the titration of 1/40. Magnification ×160.

Direct and indirect immunofluorescent tests were carried out (Beutner *et al.*, 1965b, 1968b; Cormane and Szabo, 1970) on the patient's skin, serum and post-mortem tissue homogenate from liver, spleen, inguinal lymph nodes and para-aortic tumour.

Homogenization of tissues and extraction of γ-globulins were carried out by the methods used by McGiven and Ironside (1968). Details of these methods will be published elsewhere.

RESULTS

Direct I.F. on patient's skin—strong intercellular I.F. on epidermis with anti-IgG conjugate.

Indirect I.F. with patient's serum and homogenate extract of post-mortem tissues as follows: Serum—+ I.F. 1/80. Para-aortic tumour extract 1/40 (Fig 1). Liver, Spleen, Inguinal node-extract—negative I.F.

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* Anti IgG produced in sheep.
Anti IgA and IgM produced in rabbit. (Behringwekeag)

Quantity of Immunoglobulins Present (mg/100 ml)

	IgG	*IgA*	*IgM*
Serum	1800	210	60
Tumour extract	780	12	Nil

All other tissue extracts produced negative results.

DISCUSSION

The patient here described received massive doses of steroid and other drugs systemically. Despite this, his pemphigus worsened and he died. The tumour homogenate extract and serum showed a high level of autoantibodies to stratified squamous epithelium. No antibodies were detected in other organ extracts. This suggests that these antibodies were not merely *vivo*-bound.

The IgG level of the tumour extract was 780 mg/100 ml, a high concentration. On the other hand, extracts from the inguinal lymph nodes, although they were lymphoid tissue, did not show any appreciable amount of γ-globulins. It therefore seems probable that the tumour tissue was producing excessive IgG type of antiepithelial antibodies. There must have been a definite " clone " of lymphoid tissue which was responsible for these antibodies, and the development of pemphigus. This would account for the fact that the patient did not respond to a high dose of systemic steroids and methotrexate.

The view held by some authorities that pemphigus is an autoimmune disease is strongly supported by the case reported here.

My grateful thanks are due to Professor J. A. Milne and Dr W. B. McKenna, Department of Dermatology, and to Professor J. R. Anderson, Department of Pathology, for their valuable advice and guidance.

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COIFFURE KELOIDS

MARGARET A. H. BAYLES

KELOIDS are very common in the African. Although usually traumatic in origin they may arise spontaneously and they are often produced deliberately for decorative purposes.

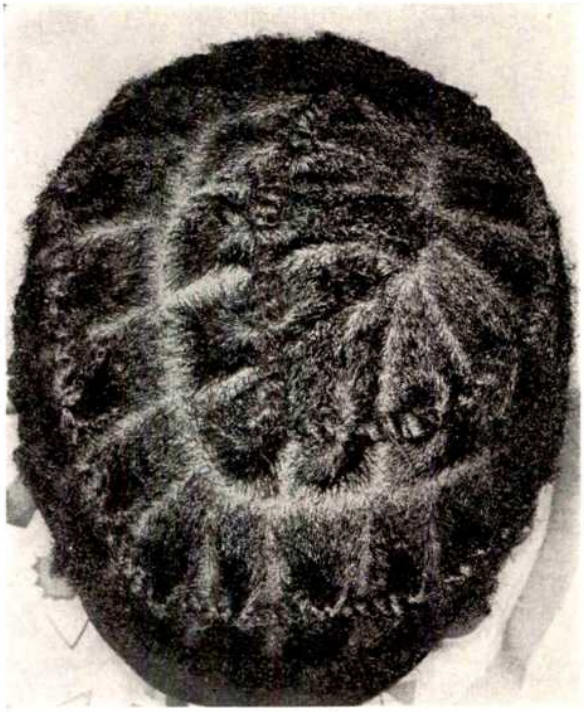

FIG. 1.—'Isibaca' hair style.

A Zulu woman, aged 34, had adopted the fashionable " Isibaca " hair style, an example of which is shown in Fig. 1. The hair is pulled tightly, twisted and plaited, exerting severe tension on the scalp. In this particular woman, gross keloids developed in the pattern of her coiffure (Fig. 2). Marshall (1964) states that " induced keloids seldom get out of hand and retain their pattern " and this is quite obvious in our patient. Reports of a similar case have not been found in the available literature.

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A histological section confirmed the diagnosis. As intralesional steroid treatment proved impracticable over such an extensive area, superficial x-ray therapy was initiated. After receiving a total dose of 600 R to one test area with encouraging results, the patient failed to attend for completion of the treatment.

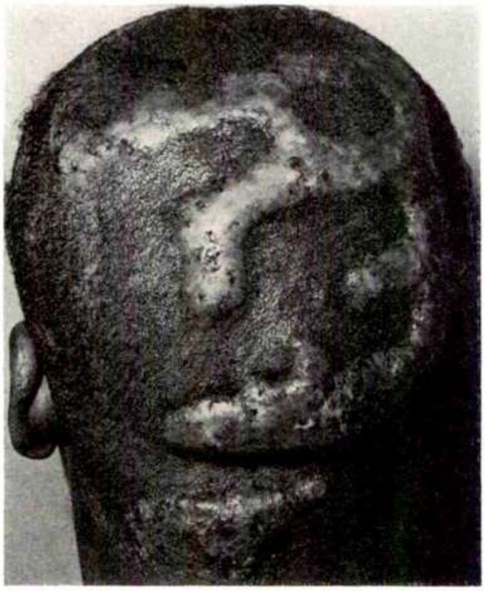

FIG. 2.—Coiffure keloids.

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Comment

PRE-CANCER AND THE SKIN

Since Percival Pott described cancer of the scrotum in a chimney sweep in 1778, and correctly attributed the disease to occupational hazard, dermatologists have signally contributed to the understanding of cancer. The emphasis has moved slowly away from the search for simple and sensational " cures " as the complexities of the neoplastic process have been successively revealed, and it is perhaps in the fields of better diagnosis and prevention that dermatology has most to offer. Until a mere decade ago, writers tended to stretch the facts upon a Procrustean bed of a single theory, to the dissatisfaction of all. It is now widely recognized that in the majority of cases several aetiological factors are at work, either successively or simultaneously (226), and an extension of this knowledge has been the definition of other areas at risk. Table I refers to the factors which are involved in the precancerous state, especially in the skin. There is very considerable overlap between these factors when specific disease processes are considered, but it forms a basis for discussion.

TABLE I

Major factors	Inheritance
	Ultraviolet light
	Irradiation
	Chronic atrophy
	Immune deficiency
	Arsenic
	Chromosome abnormalities
	Site of predilection
Minor factors	Naevi
	Viruses
	Keratoses
	Topical carcinogens
	Chronic oedema
	Trauma, and burns
	Chronic infections and ulcers
	Systematic co-carcinogens
	" Poor hygiene "

Inheritance

Recent reviews have indicated the better definition and classification of congenital and inherited diseases (145, 168), as well as showing the interesting overlap with deficiencies of the immunity mechanisms (87, 190). Examples of the latter include ataxia-telangiectasia (2, 73, 99, 190, 203), and the Wiskott–Aldrich syndrome (190, 264), both of which are well-known to dermatologists. The effects of u.v. light are enhanced in albinism (9, 111), the interesting hypopigmentation of the Chediak–Higashi syndrome (66, 275), and in xeroderma pigmentosum (146, 254). Variants of xeroderma have been described, perhaps due to in-breeding, where albinism and mental defects have occurred as well (204). It is not fully realized that malignant melanomata are also frequent in xeroderma (116, 147), besides basal and squamous cell epitheliomata. The fundamental defect in xeroderma is now better understood, and the abnormalities in nucleic acids produced by u.v. light in these patients have been partially defined (50, 77, 247).

The association of leukaemia with Down's syndrome is familiar to all physicians, but other abnormalities of chromosome number and structure are also associated with both skin changes and neoplasms (65, 70, 229). Bloom's syndrome (29, 137, 233) and Fanconi's anaemia (88, 258) are also of dermatological interest, while Klinefelter's syndrome is associated with a tendency to develop leukaemia (150), as well as vascular changes, stasis dermatitis, and leg ulcers (191, 268), which could also be precursors of squamous cell carcinoma. The increased incidence of malignancy in relations of patients with these chromosome defects, is of both interest and importance. The combination of inheritance with atrophy is premalignant in the cases of Rothmund–Thomson disease (215, 278), poikiloderma congenitale (28, 212), and in epidermolysis bullosa to a lesser extent (9, 211, 251). Certain abnormal and inherited hypertrophies are also premalignant, and neurofibromatosis is familiar, although the malignancies very rarely involve the skin. Dyskeratosis congenita (37, 52), tuberous sclerosis (59, 120, 220), and the basal cell naevus syndrome are also premalignant. The latter has been referred to as the fifth phacomatosis, because of the retinal lesions which have been observed (110). Many reports confirm the occurrence of numerous cutaneous cancers in patients with the basal cell naevus syndrome (7, 51, 98) and its inheritance has been defined as an autosomal dominant with high penetrance and variable effects. It is of great interest that a case has been reported with both the basal cell naevus syndrome and Turner's syndrome (8), so it is possible that a chromosomal defect is important in this disease as well. The various inherited polyposes of the gastro-intestinal tract have also been discussed as precursors of malignancy (23, 180), and in the case of Gardner's syndrome, the skin cancer may be a late expression of the syndrome, or else due to the enhanced effect of u.v. light (143).

There are inherited aspects to the aetiology of malignant melanoma (6), as well as the unilateral naevus syndrome (27). Skin precursors of malignant melanoma include the precancerous melanoma of Dubreuilh (71, 169), Hutchinson's lentigo (165), and xeroderma pigmentosum (116, 147, 254). Giant hairy naevi have a significant chance of developing malignant melanomata (96, 188, 242), but so can smaller ones (202), and the hairy naevi can be associated with blue naevi, which may themselves evolve into melanoma besides being locally malignant (202, 245). The naevus sebaceus of Jadassohn may also undergo malignant change (25, 158). In these conditions it is evident that the time factor is extremely important and variable, which explains the difficulty of assessing the risk for particular patients. Time is an important parameter also in cases of urticaria pigmentosa, where the cancer risk in general is small. The later the age of onset, the greater the risk of systemic mastocytosis, which may then terminate in leukaemia (74, 123, 138, 179).

Ultraviolet Light

Although malignant melanoma is a sequel both of albinism and of xeroderma pigmentosum, it is also a frequent neoplasm among Africans (176, 239), so the presence of pigment does not necessarily protect against damage. In general, however, statistical studies indicate that people with pale skin and fair hair, who spend more time than average out of doors, are those who tend to develop cancer of the skin (33, 84, 89, 123, 254) The importance of sunlight in the aetiology of skin cancer has been emphasized all over the world, as typified by reports from America (30, 149, 235), Ankara (209), and Australia (22), and it is also apparent that elevated temperatures increase the carcinogenic effect (85, 235). Some persons who would seem to be ideally cancer-prone are nevertheless spared (149), and other factors which may be involved include the ethnic background and clothing customs (209). Moisture, ozone and chemicals in the air may also be significant, as may altitude, social and work patterns, as indicated by the exceptionally high incidence of skin cancer in Tucson (235). It also seems that the ability of the skin to defend against u.v. light depends as much upon the degree of dispersion of melanin in the skin, as upon the amount of pigment produced (184). The keratoses produced by age and sun exposure are well known to be premalignant (195, 251, 257), so it is unfortunate that social pressures often force the fairer members of the community to seek a suntan, which may have a heavy but delayed price. It has recently been recognized that the keratoses of porokeratosis can also be premalignant Disseminated superficial actinic porokeratosis is probably not uncommon, and it can lead to malignancy (48, 195), while the rare porokeratosis of Mibelli does not present a significant risk, as only 2 reports have appeared of neoplastic degeneration in these lesions (122, 231). It is of interest that the keratoses of Darier's disease are virtually never followed by malignancy, despite the combined insults of chronic infection and trauma (9).

Sunlight is the cause of chronic actinic cheilitis, described in California (13), and known to be the major pre-cancerosis of the lips (181, 209). Sunlight is also the main factor in the development of squamous cell carcinoma in cheilitis glandularis, which leads to exposure of the inner and normally protected mucosal surface (161, 251, 265, 273). Cases of skin cancer are known to be associated with lupus erythematosus, and the development of epitheliomata in exposed scars is partly due to the action of u.v. light (172). This tendency is seen to a greater extent in lupus vulgaris (24, 172, 232), although lupus alone can also predispose to skin cancer (108, 183). In mice at least, experimental work has shown that a co-carcinogen is usually needed before there is a significant evolution of sunlight, changes into neoplasia (81). What nature does not always include, man all too frequently adds, as will be discussed below.

Irradiation

Post-radiation atrophy and pigmentary changes are common sequelae of treatment, whereas formerly they mainly followed accidents. It is something of a paradox that the state following treatment for cancer is itself pre-neoplastic (123, 153, 225, 278). It has been suggested that the variable evolution of these scars and lesions is a product of many co-carcinogens acting over many stages for a prolonged period of time (53). Trauma, repeated small doses of irradiation, hormones, virus infections, chemical irritants, and of course the inherited background of the patient all interact with the initial radiation effects. It has been observed that chemical carcinogens tend to promote carcinomata while radiations tend to promote sarcomata (93). This agrees with the reports of fibrosis (210), neurofibromata (67), and sarcomata (223, 230) seen after radiation. Lupus vulgaris was formerly treated with radiation, and part of the excess of neoplasms in this group of patients over those with lupus erythematosus may reflect the additive effects of 3 co-carcinogens, whereas the latter has only the scarring plus the effect of light. It is sobering to consider the number of patients with benign conditions for whom the treatment has had neoplastic consequences, including acne vulgaris, warts, ringworm, psoriasis and even " undiagnosed benign conditions of the skin " (153, 225, 263). Grenz rays have also been implicated on a few occasions (34). So, in view of the apparently additive effects of carcinogenic agents, all therapeutic modalities should be used with great caution.

Immune Defects

Ataxia-telangiectasia and the Wiskott–Aldrich syndrome have already been mentioned, but genetics and immunity are widely inter-related (87, 197). The importance of the skin as a lymphatic organ is not widely recognized (80), although the relation between immunological aberrations and lymphomata is established (26, 166). Cellular immunity is an important factor in infections with herpes (92) and with oncogenic viruses (69, 238). There is also a suggestion that human myelomatosis may be transmissible in immunologically deficient mice (170), so the associations are likely to be expanded and enlightened in the next few years. Lupus erythematosus is associated with systemic neoplasms (55, 94, 182), as well as with cutaneous cancers (172). As immune deficiency is so closely linked with lymphoma (166), it is not surprizing that malignancy is also a feature of the long-term use of immuno-suppressive drugs (207, 269). There may be a compound risk of using such drugs in patients with auto-immune disease, and scleroderma too is known to be linked with an excess of neoplasms (94, 180). Perhaps more patients are surviving the acute threat of infections only to succumb to the more chronic one of malignancy. The use of cytotoxic drugs in psoriasis is also accompanied by reduction in immunological competence (78), whereas in other psoriatics there was no abnormality (136). A recent report of the development of a lymphoma in a psoriatic treated long-term with Methotrexate is alarming (236). In this case the lesions regressed when the drug was discontinued, as with the familiar pseudolymphoma seen with sensitivity to diphenylhydantoin used in treatment of epileptics. However, it is not unknown for this pseudo-lymphoma later to become the genuine article, and we may reserve the same suspicion about Methotrexate.

On occasions, chronic candidal infection can be premalignant, so it is of great interest that abnormalities of lymphocytes and immune protective mechanisms have been found in such patients (42, 267). This is another striking demonstration of co-carcinogenesis, where one agent needs another to produce the lesion.

Lymphoid lesions *per se* are not devoid of a potential for mischief. The so-called lymphadenosis benigna cutis can evolve into a lymphoma (15, 228), and even the benign infiltration of Jessner, can, with trauma, become malignant (121). Lymphocytoma can develop into neoplasia (187, 228), and it seems that this is also true of lymphomatoid papulosis, which was thought to be a pseudo-malignancy (148). Lichen planus, especially when chronic, and hence exposed to trauma, light and infections, can evolve into squamous cell carcinomata (119, 134, 152, 163, 251, 272), and this is best known with the hypertrophic mouth lesions, where dentures and tobacco may also contribute (272). A critical review found 75 cases of lichen planus which had become cancerous in the literature, and indicated that the incidence overall cannot exceed 1% (221). The Sézary syndrome is well known to have sinister potential, even though there is a diversity of opinion as to its true nature (240). Its course is unpredictable, like mycosis fungoides of which it may be a variant (262). It is established that the Sézary cell is an abnormal lymphocyte (58), and that although the natural history may be prolonged (83), leukaemia or lymphoma may result (140). Another precursor of mycosis fungoides is parapsoriasis *en plaques* (123, 128, 139, 228), and here too the course may be very prolonged. Poikiloderma is another accepted premalignancy of the skin (178, 180, 228, 278), even though the patient may die from old age before the neoplasm is manifest.

Site of Predilection

One determinant of the tendency towards neoplasia in a tissue is the normal level of mitotic activity. When it is high, *ceteris paribus*, an optimal environment exists for the expression of carcinogenesis. In practice this means that the mucosae of the body are especially at risk. Such is the case with the penis, where the classical pre-cancerous condition is erythroplasia of Queyrat (200). This sinister entity can be treated with topical antimetabolites if recognized early, but if allowed to mature, the resulting squamous cell carcinoma has a very poor prognosis (156, 276). The Queyrat lesion is closely related to Paget's disease, which can also involve the penis (41, 123, 127, 159, 164, 195). Balanitis xerotica obliterans is equivalent to lichen sclerosus et atrophicus, and it is also premalignant (141). Penile warts can on occasion develop into true invasive neoplasms (175), and florid papillomatosis (63) and the Buschke–Loewenstein tumour (40, 159) can be locally malignant. There is a continuous spectrum among these lesions, and it is extremely difficult to say with any certainty where the definitely cancerous begins. In all penile lesions, lack of hygiene, and the chronic infection and minor trauma associated, predispose to epithelial metaplasia and neoplasia (159, 234). In this connection it has been noted that carcinoma of the penis occurs at an earlier age in patients with syphilis than those without (159, 234). However, in one case of a man aged 24 with advanced carcinoma of the penis, tight phimosis and poor hygiene had also been chronic factors in addition to syphilis (64). Although syphilis may act as a co-carcinogen, as may smegma and its products in both sexes, a careful analysis has shown no excess of cancers among syphilitics (216). It is established that infantile circumcision is accompanied by a significant reduction in subsequent carcinoma of the penis, although there are contrary views (198). There is more general agreement on the subject of penile horns (95, 195, 261). These curious lesions can grow very rapidly, seem to have a bias for men of German extraction (261), and can lead to death from metastatic carcinoma (95).

In the case of the vulva there is no doubt that the major precancerous condition is lichen sclerosus et atrophicus (16, 141, 270). This disease may apparently heal with atrophy, or develop into either kraurosis or leukoplakia (16, 18, 250). The evolution does not reduce the malignant tendency, however much various authors may dispute the terminology, and it is also established that leukoplakia arising *de novo* can evolve into cancer (266, 271) Extra-mammary Paget's disease can involve the vulva (131), as may naevoid conditions and pre-melanomata. Scars from radiodermatitis and lupus vulgaris are occasionally found in the perineum, and these may be premalignant. Perhaps this is due to the additive effects of trauma, infection and " poor hygiene ". Viral warts can degenerate into malignancy (47), but this is undoubtedly rare, although the precise incidence is unlikely ever to be known accurately because the " typical " wart lesion does not get biopsied. The more chronic form of granuloma inguinale can result in invasive epitheliomata, but this evolution is best known in areas where syphilis, poor hygiene, malnutrition, iron deficiency and other possible co-factors are also present (4, 45, 54, 129).

The oral mucosa is also peculiarly prone to malignant change, and here a combination of aetiological factors is the rule. Although it is known that chronic candidosis can occasionally result in squamous cell carcinoma (46, 79, 274), it is significant that immune deficiencies contribute to the chronic cases (42, 267). It is even more significant that many people have chronic candida, but never develop leukoplakia nor any other lesions. With lichen planus too, the influence of accessory factors such as the trauma from dentures, sharp edges of teeth, or fillings, can be important (134, 152, 272), while the use of tobacco, or a coincidental deficiency of iron or of a vitamin may also be a turning point away from normality (56, 249). Tobacco is known to be injurious to the oral mucosa as to many other tissues (86, 249), and this damage may be compounded by heat injury (56). Betel nut is used by many peoples and they have a high incidence of cancer of the mouth. The nut is often eaten or chewed in a mixture with tobacco, lime, and various chemicals, so it has been argued that these additives were the source of harm to the mucosa. When the cheeks of hamsters are painted with a dimethyl-sulphoxide extract of betel nut or tobacco separately, a high incidence of leukoplakia ensues, but if extracts of both are used, then tumours result (253). All these factors may also be associated with the development of papillomata. Although in the oral cavity, papillomata are frequently premalignant, this is of such a low order that prompt excision is followed by an excellent prognosis (5, 199, 243). There have also been reports of spontaneous disappearance of papillomata when the triggering cause has been removed, and if squamous cell epithelioma does develop, it is of lesser invasiveness than after other, perhaps more widely disturbing, agents. Leukoplakia in the mouth may be due to trauma, smoking, and many other factors all of which show different tendencies towards neoplasia (243). Syphilis was said to cause 20% of cases of oral leukoplakia (62) and while this is most unlikely to be true, occasional cases are still seen, and others may well be missed (43). An extreme rarity among mouth lesions, again indicating the multifactorial aetiology of such epitheliomata, is the occurrence of squamous cell carcinoma of the gums in the syndrome of Fanconi's anaemia (157), which was discussed among the chromosome defects.

The perianal area is also subject to the combined effects of trauma, infection and time. A few reports indicate that perianal warts can become malignant (185, 244) but these are only a minute fraction of the total. Paget's disease can involve the perineum, and sometimes this marks a deeper and evolved neoplasm (104). Chronic infections can lead to a Marjolin type of change, and I have personally seen 2 cases of squamous cell carcinoma arising in chronic peri-anal blastomycosis, which is not widely reported (9, 62). However, perineal involvement is one of the least common sites for this disease, and an extensive review mentioned no neoplasms in other parts of the body which could be attributed to blastomycosis (277). Many ulcers in this region are stigmata of neurological lesions, which may preclude survival adequate to assess malignant potential. The banal pilonidal sinus can very rarely become epitheliomatous (36, 219), but the incidence is minute. Lymphogranuloma venereum in this region may be premalignant, but with other entities it is not possible to be sure because of the paucity of reports, and the large number of co-factors also present in the patients described (9, 45).

Viruses

Warts are extremely common and rarely excite interest in the clinician. Nevertheless, a continuous spectrum exists between the giant condyloma which may be locally malignant (20, 40, 63) especially in the mouth or genitals, through florid papillomatosis which may involve the same areas (126, 227, 260), to verrucous carcinoma, which some authors group with the Buschke–Loewenstein tumour (56, 133). Common warts may also develop into malignancies, usually in a mucosa as mentioned above. Florid papillomatosis, giant condyloma, and also epidermodysplasia verruciformis are all caused by the wart virus, and all of these conditions are premalignant (57, 118, 218, 222). It is of notable interest that as epidermodysplasia evolved into Bowen's disease, the virus-like particles could no longer be detected (118). As with the Shope papilloma virus, incorporation of the foreign DNA into the host cells means that the trigger of the subsequent neoplasm is no longer visible. Inclusion bodies have also been found in kerato-acanthomata (35, 259), and these lesions can be locally recurrent and destructive, especially the multiple familial variant (144). Exposure to oils, other irritants, and to trauma have been followed by squamous carcinomata in these " benign " lesions (38, 145, 214). Recurrence is always a possibility with kerato-acanthomata, and even allowing for the imperfections of some biopsies, a potential for malignancy must be suspected. Acrodermatitis chronica atrophicans is another viral disease (100, 107), and it is a major predisposing skin lesion to squamous epithelioma of the lower limbs, especially in Germany and Scandinavia (32, 97, 186, 254, 255). It has also been associated with the appearance of skin lymphomata at the site of chronic lesions (32, 97). It has often been observed that infectious mononucleosis produces cells which are morphologically malignant, mainly resembling Hodgkin's disease, but the possibility also remains that this common viral infection can on occasions be a true co-carcinogen (1). Vaccination scars, although extremely common, can very occasionally predispose to epithelioma (205, 281) or to malignant melanoma (151). It seems likely that these rare cases already had some abnormality at the site of vaccination before the virus was introduced. Nobody would claim that vaccination predisposes to skin or any other form of cancer, yet the scar may be just a fraction more at risk than surrounding tissue. Of wider importance is the association between herpes virus type 2 and carcinoma of the cervix (12, 92, 201). This virus is venereally transmitted, and although its role has not yet been definitively assessed, it is likely to be significant. The importance of viruses in carcinogenesis is now recognized (69), and further discoveries may lead to prevention or more timely recognition of many cases.

Keratoses

Actinic and senile keratoses have already been mentioned, as have the two forms of porokeratosis. With the localized excrescence of cutaneous horns, irritation, trauma and chronic infection are important accessory factors, and the epithelial hyperplasia *per se* is only a minor component of the lesion. Malignant change is frequent with cutaneous horns (19, 62, 91), and the effect of u.v. light is significant. Seborrhoeic keratoses are usually benign, yet a few show very suspicious histology, and occasional reports of epithelial change are encountered (62, 173). This applies to far less than 1% of all lesions, and I have never seen it personally. It is more likely that these common lesions cover such a large area that chance would determine the occurrence of skin neoplasms in some overlapping portions. Besides, the opinion of a histopathologist cannot be better than the material he is asked to report upon, and inadequacies in this field are all too frequent.

Bowen's disease is another scaling keratotic lesion which is one of the best-known of the pre-cancerous conditions of the skin (101, 118, 124, 189, 251). It can be associated with arsenic (280), actinic keratoses (257), seborrhoeic keratoses (173), epidermodysplasia verruciformis (118) and other histological contexts. A virus is probably implicated, but many other aetiologies may also be invoked. It seems to be a cancer *in situ* rather than a truly pre-neoplastic condition, as it has already evolved out of normality (142, 174, 195). Extra-mammary Paget's disease is similar, and often the cells in the infiltrate can be defined as components of the underlying neoplasm (102, 103, 104, 164, 174). The so-called premalignant fibroepithelial tumours of Pinkus (142, 192) also belong to this group of early cancers. The classical site is the lumbo-sacral region, but other parts of the body can be involved, even the thumb (112). These lesions are really uncommon variants of basal cell epitheliomata (142, 192), rather than pre-cancerous entities. These exceptions point to the general rule that keratosis is less often associated with a tendency towards cancer than is atrophy. Another exception is the fascinating Werner's syndrome, where severe atrophy and premature ageing are features, as are chronic and refractory ulcers of the feet and legs. Although 10% of these patients have sarcomata or meningiomata, skin cancer has never been reported in any patient with this disease (75).

Another case of wide interest is psoriasis. Squamous cell carcinoma in psoriatic lesions is a definite rarity (114), as only 18 cases had been reported by 1921, and about 50 by 1968 (135), while most reports consisted of one case with a review of the literature (106) In the bulk of these cases there can be little doubt that arsenic was the major cause, as first noted by Hutchinson (115). Another possibility is that the psoriasis is present purely as a Koebner response around the developing epithelioma (39). Other treatments may contribute, such as excessive use of u.v. light, but the lack of such reports in the literature is perhaps comforting. Other treatments bring other risks—suppression of immune responses has been noted with cytotoxic drugs (78), and chromosome breaks have been seen after anti-folates (224). Topical steroids, especially under occlusion, can lead to atrophy and immuno-suppression, so perhaps an assembly of co-carcinogens has been effected which will produce a crop of skin cancers for the next generation of dermatologists. It is well known that tar and tar products have carcinogenic potential, and that the development of neoplasms can be increased by other co-carcinogens such as u.v light (81). Cases of iatrogenic tar tumours are extremely rare, but have been reported with psoriasis (213) and also with lupus erythematosus (217) Dithranol has similar potential (256, 282) and related compounds react with nucleic acids in the skin (31). Tars and their distillation products provide serious occupational hazards for many classes of industrial worker (68, 90, 109, 132). Prolonged exposure is important, as the latent period for the appearance of cancers of the skin has a peak after 20 years (109) It is likely that many cases also reflect the effects of trauma (68, 132), and recurrent injury and infection are important. The exfoliation which is such a feature of the increased epithelial activity in psoriasis, probably protects the patient from both infections and neoplasms. The bulk of evidence at present is that psoriasis, by itself, does not predispose to cancer of the skin.

Systemic Co-carcinogens

Historically and quantitatively, the most important agent in this class is arsenic. Hutchinson described arsenical skin cancer and the preceding keratoses in psoriatics (115), but arsenic has also been used in the treatment of epilepsy, acne vulgaris, multiple sclerosis, anaemia, and many other conditions where a " tonic " was needed. Accidental and industrial exposures also occur (60), but it is a sad fact that these cases are far fewer than those produced by treatments. Arsenical keratoses can look very like Bowen's disease, and it is possible that the aetiology of both overlaps (280). It is also well documented that neoplasms of many other sites can be found (248), amongst which is angiosarcoma of the liver, which has also been reported in a psoriatic after chronic treatment with Fowler's solution (206). Arsenic can lead to cirrhosis (206), so the use of any other potentially hepatotoxic agent becomes of increased hazard. Liver damage is well recognized with antimetabolites, and it is disquieting that chromosome changes have also been reported after folate antagonists (224). Unwanted drug effects are of daily occurrence, and many of these have at least a theoretical potential for contributing to neoplastic development. Cases have been reported with chronic abuse of a phenacetin compound leading to renal and bladder carcinomata (10). The occupational bladder cancer of dye workers is often due to naphthylamine, but many other amines and related compounds can be activated to produce carcinogens (167). The lymphoma-like picture seen with Methotrexate (236), may be a warning of other clinical conditions to be expected from sensitivity reactions, or to immunosuppression in the patients. Photosensitivity is a frequent adverse drug reaction, and this might be expected to alter the risk of patients exposed to u.v. light. Griseofulvin may occasionally act as a co-carcinogen (17), but the lack of knowledge about most of the other drugs in the pharmacopoeia is not a cause for satisfaction. Multiple factors are important in the carcinomata seen with the Plummer–Vinson syndrome, which is especially pre-cancerous for the mouth and larynx (3). As iron deficiency so greatly alters the mucosae and epithelia, it may be one factor influencing many other carcinogens, and a pointer to other metabolic parameters which have not yet been recognized. Hormones are known to affect many neoplastic conditions both benign and malignant. Part of the growth spurt of pregnancy is reflected in the enlargement of many tissues of the body, and also of papillomata, skin tags and other benign tumours. One unique case reported the development of a gingival sarcoma upon the more usual epulis of pregnancy (171), but much better known is the especially grim behaviour of malignant melanomata in pregnancy.

Chronic Oedema

The development of invasive and fatal angiosarcoma in post-mastectomy oedema has been known for over 20 years (252). This is not due to any residue of the original tumour, nor is it a retrograde metastatic process, but it is a true new growth in a previously normal tissue (246). Further support for this interpretation comes from the occurrence of angiosarcoma in chronic congenital lymphoedema, although this is a much less common event (82, 154, 160). Chronic oedema of other origin can end in the same uncontrolled fashion (14, 61, 72) so the tendency seems widespread. It is still not certain, however, whether the abnormal cells come from the walls of the blood vessels or of the lymph channels of the affected limbs. Analogy with elephantiasis would suggest that the lymphatics are likely (154), but in the absence of facts one is free to hypothesize. The stimulus involved in this new growth has not been defined, although analogies can be drawn with Kaposi's idiopathic haemorrhagic sarcoma. This is known as primarily a skin disease, but it is probably a multi-system lymphoblastoma which can involve any part of the body (76). It can evolve into a generalized neoplastic process, but there is in addition a significant excess of malignant lymphomata among patients with Kaposi's sarcoma even in the absence of obvious systemic spread (155, 208). When the cause or causes of Kaposi's sarcoma have been discovered, it can be expected that this knowledge will also be applicable to the neoplastic potential of chronic oedema from other causes. A case of Kaposi's disease has been reported at the base of a cutaneous horn (91), so it is possible that there is some overlap with trauma and even u.v. light, as in many other pre-cancerous conditions.

Trauma and Burns

Marjolin described the development of skin cancers after gunshot wounds, fractures, and avulsion injuries in his *Dictionnaire de Médicine* in 1828. Although he did not specify burns or burn scars in his list of cases, many of Marjolin's patients would have had considerable heat injury as well. Recent and fresh burns can also lead to malignancy (11, 33, 113), so it is important not to compound these injuries by irradiation. In the case of chronic burns, frequent infections and irritation are also important, and experimental work suggests that a co-carcinogen is needed for the development of malignancy (11). About 18% of all malignant tumours of the skin occur in scars, and about 38% of epitheliomata of the hands

(11, 33). Sarcomata and basal and squamous epitheliomata have all been reported after burns (11, 33, 254), and sarcomata have also been described in scars (125, 223) and after lupus vulgaris (24, 108, 183, 232). The lack of normal tissue may enhance the carcinogenic effect of radiation, of u.v. light and chemicals, as well as predisposing to infection. These factors no doubt also contribute to the carcinogenic potential of atrophic processes already discussed. Other kinds of trauma are difficult to evaluate. Occupational hazards are magnified by frequent repetition, and it is often difficult to separate the contributions of each insult to the final neoplasm. Everybody is daily exposed to minor injury, and *post hoc* reasoning may subsequently link unrelated events, especially if a compensation case is involved. Yet, in industry at least, trauma seems to have a co-carcinogenic effect (68, 132), and a neoplasm has even been attributed to a single injury (279). In the discussion of the legal process arising out of this case, one witness said that 2% of all sarcomata could be attributed to injury, while in a survey of squamous epitheliomata, trauma seemed significant (33). For oral pathology, repeated insults from dentures, nervous biting habits, and chemical and thermal damage are crucial. The skin cancers after vaccination, both epitheliomata (205, 281) and melanomata (151), may owe more to the trauma of the procedure than to the virus introduced at the time. This consideration may also apply to tattoos, as this very common custom is accompanied by many complications, but the rarest of these is subsequent neoplasia (21, 130). One unusual case was the malignant melanoma which developed at the site of an accidental tattoo in a child with the tip of an indelible pencil (241). Trauma is a major determinant in the plantar malignant melanoma of Africans (176, 239), and it can lead the benign lymphocytic infiltration of Jessner to evolve into a malignancy (121). Everybody receives trauma, but only a fraction of those injured develop skin cancer, even if there is a pre-cancerous condition present as well. Hence it is clear that trauma is only a minor factor, and it is only rarely of crucial significance in the appearance of cancer of the skin.

Chronic Infections

Virtually all the infections which are said to have been followed by neoplasia are granulomatous processes, capable also of giving rise to pseudo-epitheliomatous hyperplasia (142). In the case of amoebiasis, pseudo-cancers are well known in many sites, including the perineum, but I know of no proven neoplasm after this disease, whereas there are many reports of unnecessary bowel resection. Syphilis can lead to pre-cancerous oral leukoplakia, and it is also possible that syphilis may be a co-factor concerned with the time of appearance of carcinoma of the penis. Gummatous ulcers have been reported to undergo a Marjolin degeneration, but these lesions are now as rare as hen's teeth. Yaws ulcers have been involved in the causation of malignant melanoma, but in these cases the native treatment involves roasting the affected limb over a slow fire of burning resin, so that heat and trauma may be more significant than the yaws (49, 162). Granuloma inguinale has preceded carcinoma in both males (54) and females (4, 45), but it is also known that squamous epithelioma can simulate granuloma inguinale (129). Lymphogranuloma venereum can sometimes be followed by neoplasia (9, 45), but here too, many other factors coexist, and it is difficult or impossible to evaluate the importance of the infection alone.

For chronic osteomyelitis sinuses and for chronic fistulae (11), there is much stronger evidence linking the infection with the later neoplasm. Pilonidal sinus on the other hand is very common, especially in minor degree, and the appearance of cancer is very rare (36, 219). Leg ulcers form another disputed group, or rather groups in view of the known multiplicity of causes. Although everybody knows that chronic leg ulcers can develop into malignancies (11, 114, 123, 255), single cases are still of sufficient rarity to appear in the literature (105, 237). Varicose ulcers are the least likely to undergo this change, probably less than 0·1% (105), but burns or underlying bone disease can increase the risk (11), as may the relation of trauma to the appearance of the ulcer (237), and treatments may also contribute. Tropical ulcers are a special case, as causes are widely varied, and include many specific bacterial, protozoal and fungal agents, and important nutritional and metabolic factors. In some parts of the world these ulcers commonly develop into fungating carcinomata of poor prognosis (44), while in others they do not. Pseudo-carcinomatous hyperplasia is familiar at the edge of chronic ulcers, and it can also occur with stasis dermatitis, pyoderma gangrenosum, and many other conditions, including basal cell epithelioma (142). So, for many reports, the lack of adequate biopsies at various stages of the illness leaves a healthy doubt in the memory.

Table II summarizes the data about many of the conditions mentioned above, and the figures refer to the percentage of patients who subsequently develop malignancy. The

TABLE II.—*Premalignant Conditions of the Skin*

100–50%
Erythroplasia of Queyrat
Xeroderma pigmentosum
Albinism
Sézary syndrome
Kaposi's sarcoma
Extramammary Paget's disease
Parapsoriasis en plaques
Pinkus' tumours

50–25%
Poikiloderma
Bowen's disease
Lichen sclerosus et atrophicus
Balanitis xerotica obliterans
Cheilitis glandularis
Epidermodysplasia verruciformis

25–10%
Cutaneous horns
Arsenical keratoses
Radiodermatitis
Giant hairy naevi

10–1%
Leukoplakia
Acrodermatitis chronica atrophicans
Lupus vulgaris
Chronic lymphoedema
Scars
Lupus erythematosus
Oral papillomata
Chronic lichen planus

Rare (less than 1%)
Granuloma inguinale
Epidermolysis bullosa
Chronic candidosis
Porokeratosis
Chronic ulcers
Mastocytosis
Warts

figures have been collated from many sources, most of which have been cited in the text. But in all cases the author's own experience has modified the data, so that he alone is responsible for any errors or omissions.

DISCUSSION

The skin is an organ which includes protection among its many functions. As such it is in the van of any assaults upon the body from within as from without. Table I listed the factors mainly involved, and many of these can be seen to involve the biochemical inheritance of the cell. It has been argued that such a change is essential for the maintenance of the neoplastic process (65). The importance of the natural defences of the body is evident from this same table, as very few factors can, in isolation, cause cancer. We still have much to learn about the details. The marked inflammatory infiltrate of pre-cancerous conditions has been thought to be an immune response (196), while it has already been noted that atrophic processes, scars, and deficiencies of the immunity mechanisms all encourage the development of epitheliomata. Recent experimental evidence indicates that the loss of tissue-specific antigens may be of crucial importance in local invasion by malignant cells (177). The difference between basal and squamous epitheliomata partly reflects relationship to the stroma, and the independence of squamous cells is shown by the invasive nature of the tumours (194). So it is perhaps significant that many of the minor factors listed in Table I can predispose to squamous cell lesions, while the major factors mainly favour basal cell epitheliomata. The presence of noxious (even if intended) chemicals in diet and external environment will possibly uncover more pre-cancerous conditions in the future, as will increased survival with antibiotics, corticosteroids, and cytotoxic drugs, often used in combinations. Nevertheless, the efficiency of the body's defences is far more impressive than its occasional failures, so I will conclude with an optimistic list of common skin conditions which are *not* linked with premalignancy. Table III would have been much longer if it had not been for the harsh fact that many other conditions can be markers of internal cancer (22, 180).

TABLE III.—*Skin Conditions Not Linked with Premalignancy*

Eczema-atopic and contact
Psoriasis
Acne vulgaris
Seborrhoeic dermatitis
Congenital ichthyoses
Most naevi
Most cystic lesions
Pityriasis rosea
Superficial cutaneous mycoses
Pemphigus vulgaris
Acute bacterial infections
Most chronic bacterial infections
Alopecia areata
Seborrhoeic keratoses

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Obituaries

REGINALD THOMAS BRAIN

R. T. Brain, consulting dermatologist to the Royal Free Hospital, the Hospital for Sick Children, Great Ormond Street, and St John's Hospital for Diseases of the Skin, died on December 10th, 1971.

There are many of us who will feel that with the death of Tom Brain we have lost a figure who was a type of paterfamilias. We were in fact students together, qualified together and subsequently followed similar hospital appointments. But he was five years older than most of us; he entered medicine after teaching and war service. He had more knowledge and experience and we naturally took our problems to him. He was born on June 6th, 1894, and was educated at Queen Elizabeth Grammar School, Tamworth. From 1914–19 he served in the Royal Army Medical Service and then went to the London Hospital as a medical student. He qualified M.R.C.S., L.R.C.P. in 1923 and took the M.B., B.S.(London) the following year. He became M.R.C.P. in 1925 and was elected F.R.C.P. in 1935.

The Deparmtent of Dermatology of the London Hospital had acquired an international reputation under Sequeira. Brain was soon attracted to this department where O'Donovan, Arthur Burrows and I were also working. He was later appointed Consultant Dermatologist to the Royal Free Hospital, the Hospital for Sick Children, Great Ormond Street, St John's Hospital, the Hammersmith Hospital and the Evelina Hospital.

He had acquired an interest in laboratory investigations while he was working in a field hospital during the War. This interest was maintained at the London Hospital through his contact with A. C. Martin, and later with Professor Bedson, with whom he carried out important work on virus diseases, particularly herpes simplex, and his contributions were widely recognized. He summarized his years of thoughtful study of the herpes virus in his notable Watson-Smith lecture to the Royal College of Physicians.

Like myself, Tom Brain was a first assistant to Sequeira who was fond of him and was anxious that he should collaborate with him in producing the later editions of his famous textbook. Brain was meticulous in this, his chief written work, and contributed most of the scientific material. It was typical of him to agree to accept no royalties for his contribution to the textbook and to allow all to go to Sequeira during his lifetime.

He was absorbed rather with routine work, the common diseases, than the less important rare frills on the fringe of dermatology. He understood and was fond of children and was at home with their parents. But he was also a good therapist and his writings on children's ills were wise and sound.

He was modest and retiring but his orations always attracted a large audience, and he was a fine teacher with a great following of students, particularly postgraduate.

He was a family man, always useful about the home and understanding and caring for his cars for which he invented numerous safety devices. He was happily married to the sister of a clergyman and had two sons and one daughter. One son is a Consultant Geriatrician in London.

He died as I think he would have wished. Coming in from gardening he fell unconscious and died in hospital within a few hours without recovering consciousness.

Our sympathy goes out to his widow and family.

J. T. Ingram

Dr John Belisario writes: "It was with very real regret that I read in the *British Medical Journal* of the passing of Dr R. T. Brain (Tom to his friends) on the 10th of December last year. I had known him for over forty years and acquired a great respect for his knowledge of all things dermatological in addition to a sincere liking for him as a friend. During all this period, in addition to our occasional meeting when I visited England, we kept up an intermittent correspondence with each other. I know that all my Australian colleagues who knew him would wish to be associated with my expression of sincere sympathy, not only to his family but to his British Dermatological colleagues in their sad loss."

ARCHIBALD GORDON SMITH

Dr A. G. Smith, former Consultant Dermatologist at the Norfolk and Norwich Hospital from 1946–63, died on January 2nd, 1972. He was 73.

Archibald Gordon Smith was born at Gourock on December 19th, 1898, and educated at Greenock Academy. He excelled in Latin and Greek, and classical history was subsequently a lifelong interest. A career in the Church was contemplated but medicine was his eventual choice, and he entered Glasgow University Medical Faculty in 1915. Like so many others of his generation his early career was interrupted by war service, and he volunteered as a surgeon probationer in the Royal Navy in 1917. He served on H.M.S. *Taurus* on the Harwich Patrol, and was present at the action at Zeebrugge. He returned to Glasgow University at the end of the war where, before graduating M.B., Ch.B. in 1920, he gained the Hunterian Gold Medal in Medicine and Surgery and the Sir William MacEwan Gold Medal in Surgery. House appointments in Surgery with Sir William MacEwan and at the Norfolk and Norwich Hospital followed, and after a spell as demonstrator in anatomy at the Middlesex Hospital he took the F.R.C.S. in 1925. There followed a 3-year period as Dean of the Faculty of Medicine at Colombo University, and 3 years in Bombay where he entered a surgical partnership. It was during this time in India, through the wide cross section of disease which he saw, that an interest in dermatology developed. His return home was hastened by malaria and after convalescence he took up the study of skin disease with an appointment at the Lambeth Poor Law Hospital. He proceeded M.D. in 1934 with a thesis on *Gold and Bismuth in the Treatment of Lupus Erythematosus*. Thereafter he took up private practice in Norwich until the outbreak of war in 1939. He joined the R.A.M.C. and the end of the war found him in Rawalpindi with the rank of lieutenant-colonel. On his return to Norwich he was appointed Dermatologist to the Norfolk and Norwich Hospital in 1946. Dr Smith was a member of the British Association of Dermatology and, after retirement, President of the Norwich Medico-Chirurgical Society.

Archie Smith was the first Consultant in Dermatology at the Norfolk and Norwich Hospital. To a detailed knowledge of the specialty his varied career added a vast experience of medicine and people, and his contemporaries had a high regard for his clinical abilities. Trained as a surgeon initially it seems that his temperament led him to a place in dermatology where he was able to practise with his infinite tolerance and patience. He was a tireless worker and his frail stature belied the way in which he applied himself as a clinician rather than a theoretician to a large hospital and private practice. He was helpful, courteous and kind to patients and staff alike, and the writer had cause to be grateful to him for help on several occasions when faced with previously unencountered rural skin problems. During his career and on retirement he maintained his interest in clinicial medicine and medical history, and to converse with him was to put into perspective the radical changes through which the profession is passing.

In a scientific age technical expertise is expected from us by our patients, but we are remembered mainly by the humane qualities which we show to them. The huge legacy of goodwill which Archie Smith left in Norwich and Norfolk is the result of his lifelong practice of compassionate tolerance and kindness.

A. W. McKenzie

Br. J. Derm. (1972) **86**, 437.

Correspondence

The Prevalence of Dermatitis Herpetiformis

Sir,—The suggestion of Wyatt *et al.* (1971) based on a postal survey that there are not less than 600–700 cases of dermatitis herpetiformis in England, Scotland and Wales prompts me to write this letter.

For some years a diagnostic index of skin cases has been kept in Northern Ireland and in 1961 this was centralized and transferred to the Department of Medical Statistics, Queen's University of Belfast, where a punch-card system was adopted Hall and Burrows (1957, 1968).

The details of cases seen at our skin clinics (20 in all) are kept in a Central Registry. For geographical and political reasons there is little or no flow of patients into or out of Northern Ireland. As Wyatt *et al.* say, the majority of patients with dermatitis herpetiformis would not, as a rule, fail to seek medical advice, and so the figures in our central index are likely to be a true reflection of the number of cases occurring in this discrete area.

Cases of Dermatitis Herpetiformis in Northern Ireland

	Male	Female	Total
1962	24	19	43
1963	27	11	38
1964	27	19	46
1965	22	15	37
1966	26	14	40
1967	22	14	36
1968	17	19	36
1969	15	24	39
1970	20	14	34
	200	149	349

The population of Northern Ireland is 1,500,000. Allowing a population for England, Scotland and Wales of 52,000,000, this would suggest that there were likely to be about 1300 new cases of dermatitis herpetiformis per year in England, Scotland and Wales. Accepting a conservative estimate of an average duration of 5 years this would give about 6500 cases of dermatitis herpetiformis in England, Scotland and Wales.

There is no reason for this region to be different medically from other parts of the United Kingdom, though there may be some special reason for a higher prevalence of dermatitis herpetiformis. I think, however, that the prevalence of this condition must be much higher than that indicated in the postal survey.

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The Prevalence of Dermatitis Herpetiformis

Sir,—I was very interested in the account of dermatitis herpetiformis (DH) given by Wyatt *et al.* (1971) and in particular their estimate of the incidence of the condition. They suggest that there are not less than 600–700 cases in England, Wales and Scotland, or about 12 to 14 per million and say that no overall estimate appears to have been attempted in the past. I can however give figures based on the incidence of DH among the British troops in the invasion force in 1944. Thanks to the organization in B.L.A. practically every soldier with a persistent skin condition was seen by one of a team of very competent dermatologists who were able to diagnose many mild cases which might have been missed by an ordinary M.O. The number of cases reported (Hellier, 1946) were as follows:

July–September	10
October–December	9
January–March	11

This gives a total of 30 in approximately 750,000 soldiers, or 40 per million. These were of course a highly selected group of men mostly between the ages of 20 and 45. A comparable figure for women in view of the 2 : 1 sex ratio, would be 20, so that one might expect an overall figure of about 30 per million. This is higher than the figures of Wyatt *et al.* but the differences may be explained by the inclusion of the mild cases in our series.

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Fixed Drug Eruption Due to Phenazone in a Hypnotic

Sir,—Dichloralphenazone is completely dissociated in aqueous solutions to give the equivalent of a mixture of chloral hydrate and phenazone. A female, aged 71, in hospital for treatment of multiple basal cell carcinomata over the lower legs, was given two 650 mg dichloralphenazone tablets B.P. on the evening of February 14th, 1970. The following morning she complained of severe irritation over the upper thighs, and on examination an erythematous circular patch (7 cm in diameter) was present over the right anterior thigh and a smaller patch over the left thigh; over the next 24 hours a few smaller patches appeared over the left thigh. All irritation and lesions faded rapidly to be only faintly visible by February 19th. With her consent, she was given 300 mg phenazone on February 21st (i.e. about 60% the amount of phenazone contained in two dichloralphenazone tablets) and within 30 minutes an exacerbation of her eruption in exactly the same areas occurred, the eruption being preceded by a tingling feeling over the thighs. Although flaccid bullae later appeared in the larger patches, the lesions settled down after a few days. Although phenazone (antipyrine) is well recognized as a cause of fixed drug eruption it is little used alone nowadays. However, Savin (1970) did report fixed drug eruption due to oxyphenbutazone (Tanderil) which is chemically related to phenazone. Dichloralphenazone (Welldorm, Fenzol) is a non-barbiturate hypnotic commonly prescribed in tablet or elixir form; it should be added to any list of current causes of fixed drug eruption.

I thank Dr P. F. Borrie for allowing me to publish this case.

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Zinc and Copper in Psoriasis

SIR,—The literature on trace metals in the plasma or serum in psoriasis is becoming increasingly confusing, so a brief summary of the present position is appropriate. Ponomareva (1966) and Greaves and Boyde (1967) reported significant lowering of the plasma zinc concentration in patients with extensive psoriasis. Similar results were reported subsequently in an American study (Voorhees *et al.*, 1969). By contrast, Withers *et al.* (1968) and Molokhia and Portnoy (1970) did not find significant lowering of the plasma zinc in a miscellaneous group of patients with psoriasis. A year ago I emphasized the importance of standardizing the surface area of involved skin, and gave data indicating that the plasma zinc concentration is normal in minimal psoriasis but reduced in extensive psoriasis (Greaves, 1971) and I questioned the validity of results such as those of the Manchester workers which failed to take this factor into account. Since our original (1967) paper the importance of other factors including recent intake of food and corticosteroid treatment on plasma zinc levels has been defined (Davies *et al.*, 1968; Flynn *et al.*, 1971). It is evident therefore that further meticulous work with careful definition of patients and experimental conditions is required.

It is therefore particularly unfortunate to read further misleading work from Portnoy and Molokhia on the relationship of serum zinc concentrations to extent of skin involvement in psoriasis (Portnoy and Molokhia, 1971; 1972). According to these data their results were obtained on patients whose extent of skin involvement did not exceed 10%. By contrast all our patients had areas of involvement exceeding 10%. Portnoy's results do not therefore support his claim that extent of disease in psoriasis is unrelated to serum zinc concentration, and indeed his data are quite irrelevant to this issue.

Portnoy objects to our recent use of control plasma-zinc values obtained in an earlier study. If he has any evidence that the plasma-zinc concentration in any defined control population has changed over the last 4 years then I, and many others interested in zinc metabolism, would be very interested indeed to hear about it.

One further point requires clarification. Portnoy has frequently stated in accounts of his serum zinc work that neutron activation analysis is the most sensitive technique available for trace element studies. This attribute does not, however, imply any greater validity of his results compared with results obtained by atomic absorption spectroscopy since the concentrations involved in this work are well within the limits of sensitivity of both methods.

An important role for zinc in psoriasis has not yet been demonstrated, but the problem of trace metals in normal and diseased skin remains an important and largely unexplored field. It is encouraging that Doctors Portnoy and Molokhia have employed methods for detecting trace metals in skin. No doubt their continuing efforts will bring the results of our two laboratories more close together.

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Turkey Diet Regime in the Treatment of Psoriasis

SIR,—It was with interest I read the article by Spiera *et al.* in the September issue of the *British Journal of Dermatology* on results following the use of turkey diet in psoriasis. Since their preliminary communication on this subject in the *Lancet* (Spiera and Lefkovits, 1967) I too have been trying to assess the use of this regime in patients with intractable psoriasis. The diet given to my patients was similar to that used by the American workers. It was initially claimed to be a low tryptophane diet.

In my series 18 patients were studied and these were divided into 3 groups of 6 patients.

Group I

Consisted of 5 females and one male subject with chronic psoriasis. The male patient had severe psoriatic arthritis as a complicating factor. For 4 weeks this group was given turkey diet only without any local applications. As a result of this one patient showed an excellent response, 3 improved, though not dramatically, 2 showed no change in their skin condition. The most rewarding facet in this group was the production of symptom-free joints in the patient with the psoriatic arthropathy.

Following the preliminary 4 week period these 6 patients then continued to take turkey diet for a further 4 months, and were now permitted to apply a dilute steroid preparation. The combination of diet and local treatment gave an excellent result in 3 patients and a moderately good result in the other 3. The patient with the psoriatic arthritis of five years' duration continued to have painless joints.

Group II

Again consisted of 6 patients with the duration of their psoriasis ranging from 1–27 years. Two of these patients also had psoriatic arthritis. This group was given turkey diet, locally applied dilute steroid ointment and each had 50 mg of nicotanimide daily because it was thought at this stage, that the diet was low in tryptophane. On this regime each member of this group showed an excellent response within one month and their psoriatic lesions cleared satisfactorily. The 2 patients with psoriatic arthritis agreed to stop all pain relieving drugs and as a result of this, over the first few weeks their joint pains were worse but eventually with diet only great relief was felt.

Group III

As doubt had been cast on the tryptophane content of turkey meat (Farber and Zackheim, 1967; Scott, 1959) it was decided to carry out a double blind trial on this third group of patients. All 6 patients were given turkey diet, 50 mg of nicotinamide daily, locally applied dilute steroid ointment was permitted and each received a specially prepared capsule daily. Three patients received Capsule A daily and the other three Capsule B daily. Following the final assessment it was then made known in fact which patients had been receiving 1 gm l-tryptophane daily and which had been receiving dextrose as a placebo. Five of these patients showed an excellent response and one a moderately good response, thus indicating that tryptophane or its lack was not an important factor. Spiera in a personal communication (1967) agreed with this finding and published this fact at a later date in the *Lancet*.

In conclusion I cannot claim that turkey diet *per se* resulted in a dramatic clearance of psoriasis in my patients. Perhaps American turkeys are constitutionally different from the Irish breed! I would, however, agree that turkey diet plus locally applied dilute steroid ointment gave a very beneficial result and the diet certainly appeared to act as an adjuvant to the local treatment and this form of therapy is well worth trying in patients with chronic psoriasis resistant to other forms of therapy.

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AGNESE KELLY

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Treatment of Hyperhidrosis with Topical Propantheline—a New Technique

SIR,—After submitting our article on topical propantheline, we learned that the manufacturers had regrettably decided not to make the aerosol available commercially. Instead a powder is to be provided for the use of dermatologists. It is hoped to submit the results of a comparison between this powder and the aerosol in the form of a letter to this Journal.

Beaumont Hospital
Slyne Road
Lancaster

R. H. SEVILLE
J. C. FRANKLAND

ADDENDUM

(Griffiths, W. A. D., Buxton, P. K. & Tan, R. S.-H. *Br. J. Derm.* (1971) **85**, 603)

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Books

Sjögren's Syndrome. M. A. SHEARN (1971). Philadelphia: Saunders. Pp. 262, 108 illus. Price £4.90.

This is the second volume in the series *Major Problems in Internal Medicine.* It is based on a study of 80 patients in San Francisco and a review of the world literature

In a historical introduction there is an interesting account of the reports leading to the establishment that Mikulicz's disease and Sjögren's syndrome are the same disorder. In the past 15 years it has been realized that widespread systemic manifestations and immunological abnormalities occur along with the previously described keratoconjunctivitis sicca, deficient secretion of lacrimal, salivary and other glands, parotid swelling and arthritis. Systemic abnormalities include pancreatitis, pulmonary and neurological involvement, and renal changes such as renal tubular acidosis, Fanconi's syndrome, chronic interstitial nephritis and defects of concentrating power.

Cutaneous manifestations include dryness, scaling, pruritus, alopecia, Raynaud's phenomenon, vasculitis and nonthrombocytopenic purpura. The disorder is not infrequently associated with systemic sclerosis, systemic lupus erythematosus, Hashimoto's thyroiditis, purpura hyperglobulinaemica, polyarteritis and polymyositis, and the age at onset pattern also indicates an autoimmune aetiology.

The syndrome and its immunological abnormalities are commoner in relatives of patients, consistent with a genetic predisposition. Women are 9 times as likely to develop the syndrome as men, suggesting that some of the inheritance factors are on the X chromosome. It is interesting that the age distribution in one series shows an initial peak at 20–29 and a second peak in the early 50's. This probably indicates that 2 different genotypes occur. Recently myxovirus-like particles have been demonstrated in the renal endothelium; if they are confirmed as viruses, they could be precipitating agents in this syndrome.

The book is well produced and the 579 references cover the literature up to early 1971. The section on cutaneous manifestations occupies only a page and dermatologists are unlikely to buy the book for this. However, those interested in connective tissue and autoimmune diseases, and all hospital and university libraries, should possess a copy as it is likely to be the standard work on this subject for some time.

N. R. ROWELL

Iatrogenic Diseases. P. F. D'ARCY and J. P. GRIFFIN (1972). London: Oxford University Press. Pp. 208, 16 illus. Price: Boards, £5.00, Paperback £3.50.

This book is almost entirely devoted to the side-effects of drugs. There is brief mention of radiation sequelae. The principal authors are respectively the Professor of Pharmacy at the Queen's University of Belfast and a Senior Medical Officer in Medicines Division of the Department of Health and Social Security. The chapter on skin diseases is contributed by A. McQueen, Lecturer in the Histopathology of the Skin at Glasgow.

The first chapter discusses epidermiological aspects of drug-induced disease; curiously no mention is made of the extensive study carried out in Belfast by Hurwitz and Wade. This is followed by a chapter on drug interaction. Thereafter, the effects of drugs on individual organs or metabolic systems are considered, largely in pharmacological terms. Immunological mechanisms might have been more extensively discussed and the difficulties of testing for drug sensitivity deserve explanation.

The chapter on disturbances of porphyrin metabolism is of particular interest to dermatologists. The section on reactions of the skin resorts to the alphabetical approach natural when a vast field has to be covered. In subsequent editions it might be as well to avoid such clinical tit-bits as the information that the rash of carbromal is strikingly similar to that of dermatitis lichenoides purpurica et pigmentosa or that a truly interesting feature of acne vulgaris is that it is only seen above the nipple line.

Lists of the official and proprietary names of drugs enable cross-reference to be made. Each chapter has an extensive bibliography and the index appears adequate. The publishers are to be congratulated on issuing a paperback edition at a moderate price: this might be further reduced by dropping the illustrations which are not entirely successful or necessary. It is both a readable book and a useful source of reference in a large subject of importance to all who use drugs or teach clinical pharmacology.

P. J. HARE

Colours for Cosmetics. 3rd Report of the Farbstoff-Komission of the Deutsche Forschungsgemeinschaft. (1971). Wiesbaden: Franz Steiner. Pp. 31.

The classification of dyes and colours is still based on national systems and nomenclature. All such lists are provisional, depending as they do on the necessarily slow accretion of toxicological and dermatological testing. Contact dermatitis due to dyes is almost certainly underestimated but patch testing is often hindered by the variety of dyes implicated and difficulty in their recognition and identification.

It is, therefore, praiseworthy of the Dye-Stuff Commission of the German Research Association to make available an English translation of the Introduction and Register of Colours from their 3rd report. Not only are all cosmetic colours cross-listed by the Schultz number and Colour-Index designation but an indication is given of their recommended limits of use. I hope that this valuable essay in international co-operation will be extended.

The small monograph can be obtained, free of charge, from Deutsche Forschungsgemeinschaft, D-53 Bonn-Bad Godesberg, Kennedy-Allee 40, West Germany.

D. S. WILKINSON

Epidermolysis Bullosa. T. GEDDE-DAHL (1971). Baltimore: Johns Hopkins Press. Pp. 180, illust. Price £9.50.

Working from 1961–69 at the Institute of Medical Genetics of the University of Oslo, Dr Gedde-Dahl carried out a comprehensive clinical, genetic and epidemiological survey of epidermolysis bullosa in Norway. With the assistance of dermatologists and paediatricians, 53 probands were ascertained belonging to 46 families, and 124 secondary cases were traced. A very high proportion of the affected individuals and of their relatives were examined. The cases were classified according to clinical criteria, which the author defines.

Ten probands from 8 families were considered to be suffering from epidermolysis bullosa simplex. Most presented the clinical features of either the Weber–Cockayne or the Koebner type of the disease but one family with blistering associated with easy bruising is classified as a new “ Ogna ” variant. The clinical heterogeneity within the simplex group appeared to be essentially quantitative.

There were 39 probands from 34 families with dystrophic forms of epidermolysis bullosa. A single family presented the dominantly inherited albopapuloid type of Pasini. The remaining dystrophic cases, apparently of recessive inheritance, are classified clinically as 9 types, of which one, the congenital, generalized, inverse, non-lethal type, has apparently not been previously recognized.

The genetic and epidemiological study of these patients is thorough and well documented, and this small volume is a valuable contribution to our knowledge of a complex group of diseases. It is unfortunate however that the work was started before electron-microscopic studies had provided reliable new criteria for the classification of epidermolysis bullosa. The site of bulla formation in the Herlitz type differs from that in the recessive dystrophic type. Once the diagnosis is established on ultrastructural criteria, clinical differences, more constant than a " lethal " or " non-lethal " course, allow the diseases and their variants to be distinguished. It is to be hoped that Dr Gedde-Dahl will determine and publish the electronmicroscopic features of the bullae in the various clinical types which he has delineated in such detail.

ARTHUR ROOK

The Significance of Physical Signs in Medicine. PETER MILLS (1971). London: H. K. Lewis. Pp. 106. Price £2.25.

"And what is the use of a book," said Alice, " without pictures or conversations ? "—Lewis Carroll.

In this instance, the lack of illustration is compensated by a lively and interesting text. The book is inexpensive, and though the presence of coloured pictures would have enhanced it enormously, one realizes that the increased cost would have made the price of the book prohibitive.

The volume is more advanced than other works on " Physical Signs ", and assumes more knowledge on the part of the reader, so that a physical sign is used to recall possible associations with such a sign, in terms of syndromes. The consideration of dermatological physical signs is well to the fore, and the dry skin of myxoedema, the pallor of anaemia, the loss of hair in hypopituitarism, and the pigmentation of Addison's disease and anorexia nervosa, all appear on page one. A paragraph on the examination of the skin might have been an opportunity to describe physical signs in dermatology in the same manner as they are later described in cardiology. However, jaundice, pigmentation and purpura gain mention, while erythema nodosum, myxoedema, thyrotoxicosis and spider naevi are also included.

The rest of dermatological physical signs are omitted and only gain mention as " the many different rashes that come under the heading of Dermatology ".

On inspection of the head and neck, rashes of the Butterfly area of the face are described in " Lupus Erythematosis ". (Pace—Lupus Erythematosus, and is the Butterfly still alive?).

We have succeeded in forcing dermatologists to be interested in general medicine, and if we work hard for the next half century, we may persuade physicians to be interested in dermatology.

This is a good book, fulfils its function well, and must be highly recommended.

MAURICE GARRETTS

Br. J. Derm. (1972) **86**, 445.

Quarterly Review

CONTACT DERMATITIS XVIII

Ancillary Aids in Elucidation of Causes of Contact Dermatitis

All dermatologists who carry out investigations into the cause of cases of contact dermatitis arrive, from time to time, at a situation of frustration when it becomes difficult, or even impossible, in practical terms, to establish the final link in the chain of cause and effect. It may well be that the proof of the clothing is in the wearing and that a positive patch to a piece of clothing can be associated with one garment alone but the patient cannot be wholly protected against future attacks unless the exact offending chemical and its distribution are known. Sometimes this is not difficult. Dermatologists are fortunate in this country in having, by and large, industrial concerns and manufacturers who will respond to serious requests for information about their products; they in return respect the confidence that is sometimes, not unnaturally, asked of them. Thus, a machinist can be protected against recurrence of a dermatitis due to MBT in a cutting oil if alternatives free from this substance are known and available.

But at times the identification of an allergen becomes tedious and daunts all but the most obsessional, e.g. in textiles and cosmetics.

Dermatologists are not usually trained organic chemists and, outside a few large centres, seldom have recourse to the specialized techniques of chromatography, infra-red spectroscopy etc. required to identify an allergen exactly. In this respect Foussereau is especially fortunate and his joint studies with organic chemists (Foussereau *et al.*, 1967; 1972) have enabled an exact determination to be made of oil of laurel and Disperse Yellow 3 allergenicity. The Contact Dermatitis Newsletters provide an increasingly useful source of reference for the distribution of environmental allergens. But the more complex these are seen to be, the more the onus on the dermatologist to achieve a greater accuracy in determining the exact allergen implicated.

Fortunately, there are a number of ancillary tests that can at times confirm the presence of certain chemicals in suspected articles. These range from the very simple to the moderately complicated. A " spot test " for nickel uses a 1% alcoholic solution of dimethylglyoxime, giving a pink or red colour in ammoniacal solution. This is inhibited when the nickel is chelated by ethylenediaminetetraacetic acid. A very sensitive test is based on the violet colour of nickel diphenylcarbazine, a salt derived from the condensation of pheylhydrazine and urea. Consequently, these tests, or modifications of them, can be used conversely to detect the presence of EDTA or hydrazines. Chromium and other metals can be similarly detected, usually in extremely small dilution, by similar procedures which can be expanded, through varying degrees of complexity, to include a number of non-metallic substances incriminated as the cause of contact dermatitis. Feigl's textbook (1960) is an invaluable guide to these.

Test papers for the identification of various metals are now available through J. T. Baker Chemical Co. and are fairly sensitive.

The chromotropic acid test for formalin in textiles is well-known and remains a useful way of " clearing " articles of clothing for use by patients sensitive to it.

Foussereau *et al.* (1972) have pointed out that Sidi's technique (Sidi, 1959) of steam heating stockings before applying pieces as patch tests, may convert a negative or doubtful test into a positive one. Nylon dyes can also be extracted for patch tests with hot ethanol for 30–60 min.

The presence of fluorescent contact sensitizers can be detected with the Wood's light. Such an examination of two samples of carbon black enabled the source of an anthracene-induced photodermatitis to be detected in a printing factory (Lindsay *et al.*, 1958). Wood's light is also occasionally useful in showing up faded or inconspicuous patch test reactions.

Even though these tests may be relatively simple they require time, patience, accuracy and some basic equipment and the first of these is at a premium in most dermatological departments. The time may come—it has certainly not come yet—when the need for a trained technician in dermatological departments devoting much time to contact allergy will be officially recognized.

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D. S. Wilkinson

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News and Notices

B.A.D. Summer Meeting, London, July 6th, 7th and 8th. Request for Papers

Papers for this Meeting are requested. They should be submitted as soon as possible, in the form of a summary of approximately 200 words. The summary should be as precise as possible, avoiding vague statements (e.g. the results will be discussed). The summary should be sent to the Hon. Secretary, Dr J. S. Pegum, 53 Philpot Street, London, E.1.

Dermatological Study and Research Centre at St. Mary's Hospital, London, W.2

A new Dermatological Study and Research Centre at St. Mary's Hospital, London, W.2 was opened by Professor Sir John McMichael on February 16th, 1972.

The Wellcome Trust generously contributed £45,000 (to include an electron-microscope for study of the skin, housed in the Anatomy Department) and the Medical Research Council £15,000 to establish the Unit. After inspecting a display of work on cell kinetics and mechanisms of drug action in psoriasis and the immunological abnormality in dermatitis herpetiformis and the coeliac syndrome, Sir John addressed the seventy-or-so guests in the Medical School. He emphasized the great need for work on the more common dermatoses causing distress and disfigurement.

Appointments

East and South Liverpool Groups

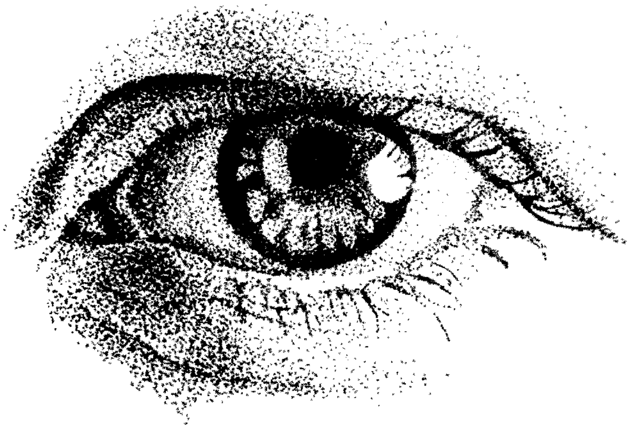
Dr J. L. Verbov has been appointed Consultant Dermatologist to the East and South Liverpool Groups of Hospitals. He qualified M.B., Ch.B. at the University of Liverpool in 1959 and after National Service in the R.A.M.C. as a Clinical Officer in Dermatology, he held Registrar appointments in Medicine at Whipps Cross Hospital and later at Guy's Hospital, London. Since 1967 he has been Senior Registrar at St Bartholomew's Hospital. He obtained his M.R.C.P. (Lond.) in 1965 and his M.D. (Liverpool), with a thesis on Dermatoglyphics, in 1971. Of many research interests dermtoglyphics has formed an important part but he has also done work on hypohidrotic ectodermal dysplasia and the use of intralesional triamcinolone.

North Staffordshire Hospital Centre, Stoke-on-Trent, and Mid-Staffordshire Hospital Group

Dr R. P. R. Dawber has been appointed Consultant Dermatologist to the above. He qualified M.B., Ch.B. (Sheffield) in 1964. After house officer appointments at Sheffield Royal Infirmary, including 3 months as house physician to the Skin Department, he spent 3 years training in general medicine occupying posts at Wharncliffe Hospital and the Royal Infirmary Sheffield, Doncaster Royal Infirmary and Worcester Royal Infirmary. After obtaining the M.R.C.P. (London) in 1968 he became Registrar to the University Department of Dermatology, Newcastle upon Tyne. In December 1969 he was appointed Senior Registrar and Clinical Tutor at St John's Hospital and the Institute of Dermatology, London and concurrent with this appointment since August 1971 he has held the post of Honorary (Part-time) Consultant Dermatologist and Lecturer in Dermatology at the Hammersmith Hospital and Royal Postgraduate Medical School, London. His particular interests are the applications of scanning electron microscopy in dermatology, vitiligo and its associations with various systemic diseases, and factors influencing nail growth in health and disease.

Correction

We regret that the legends of Figures 3 and 4 were transposed in the article entitled " Histochemical Applications of Skin Surface Biopsy ". (*Br. J. Derm.* (1972) **86**, 20.)



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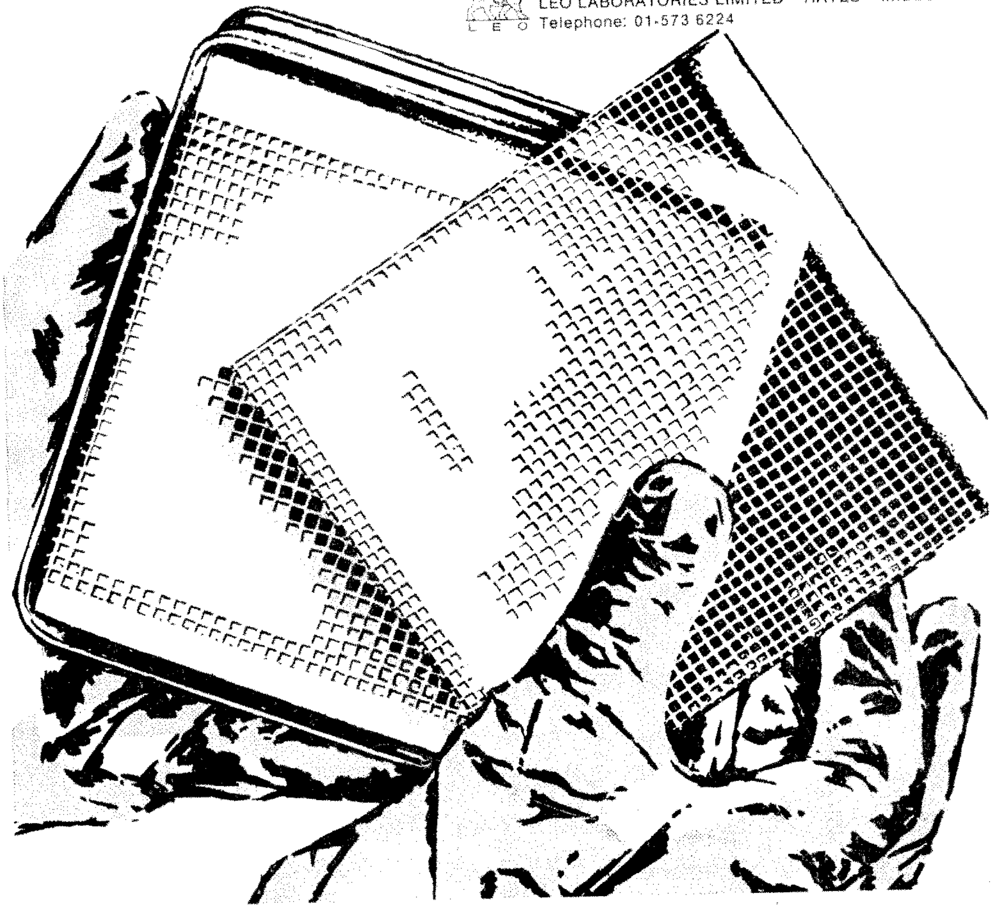

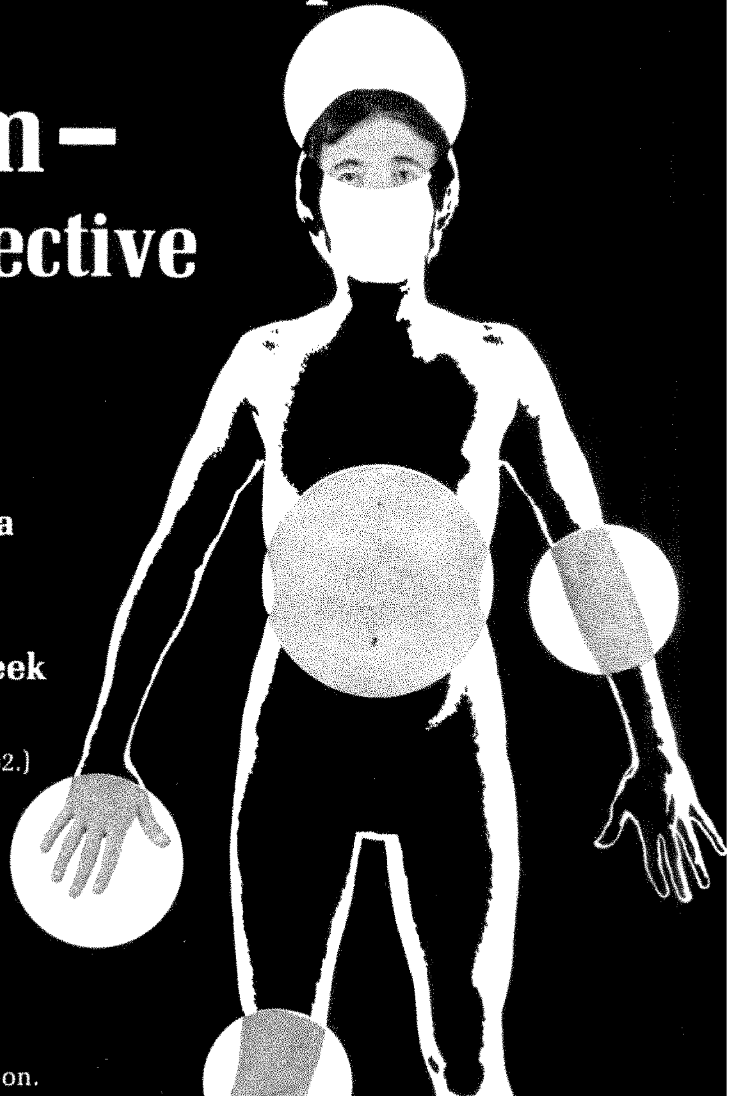

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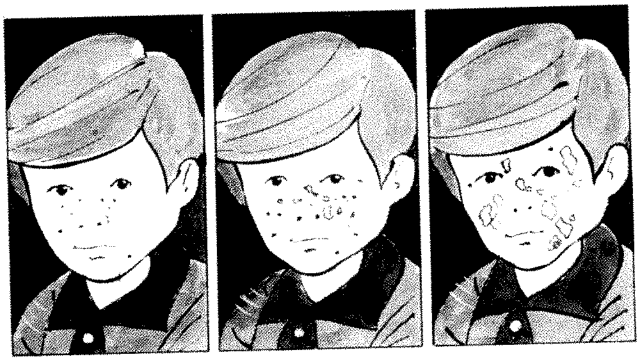

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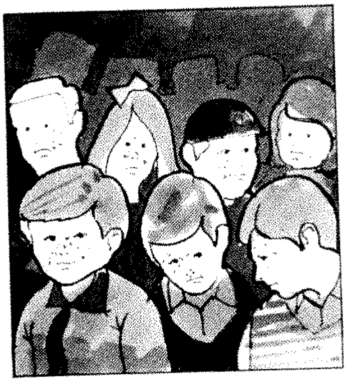

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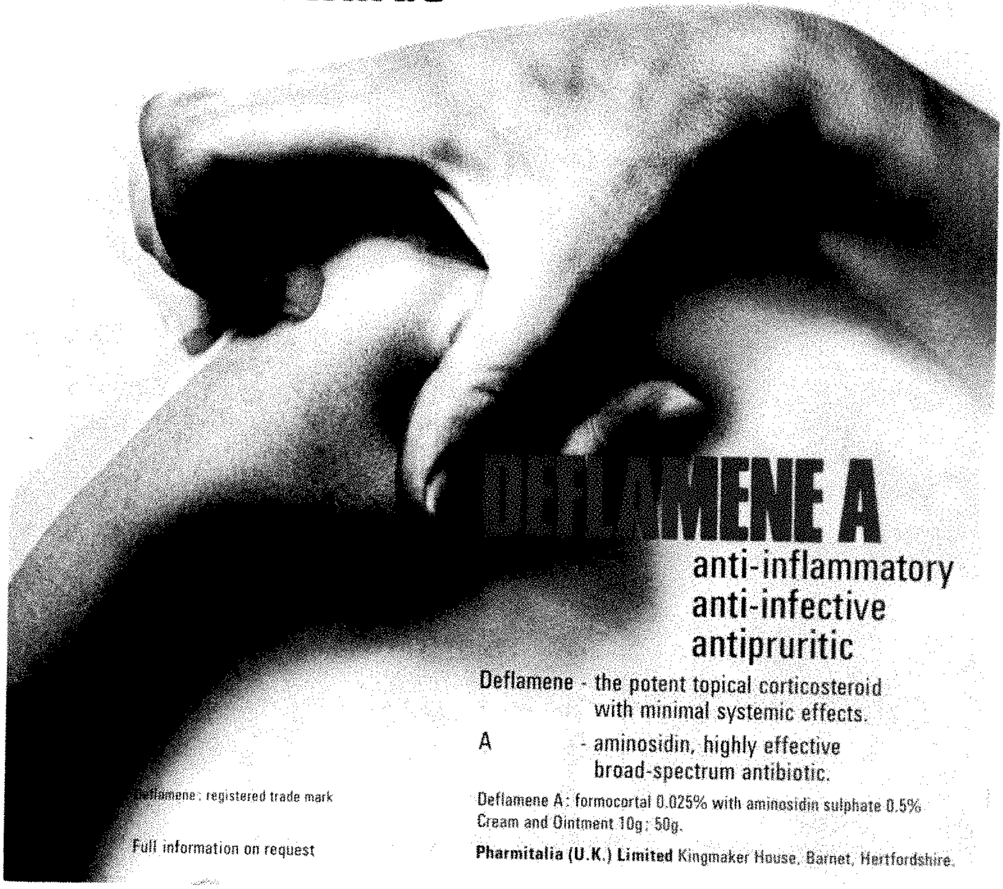

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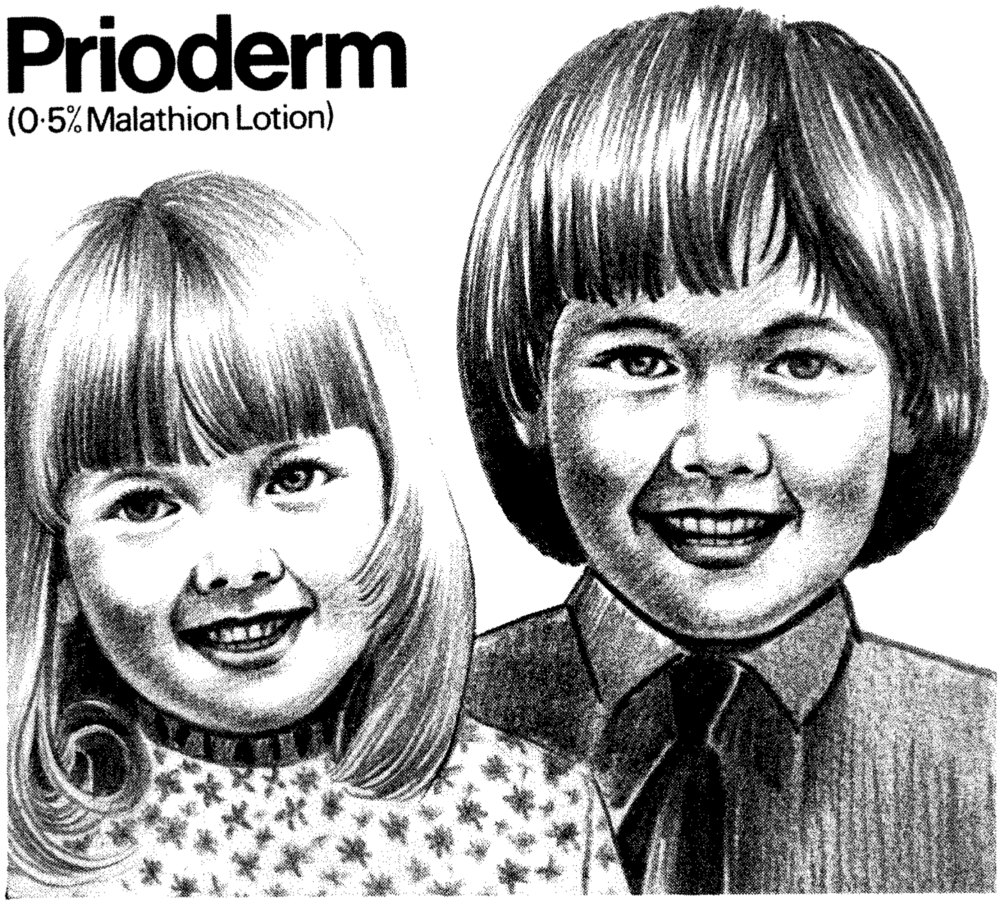

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Community Medicine (1971) 126.145

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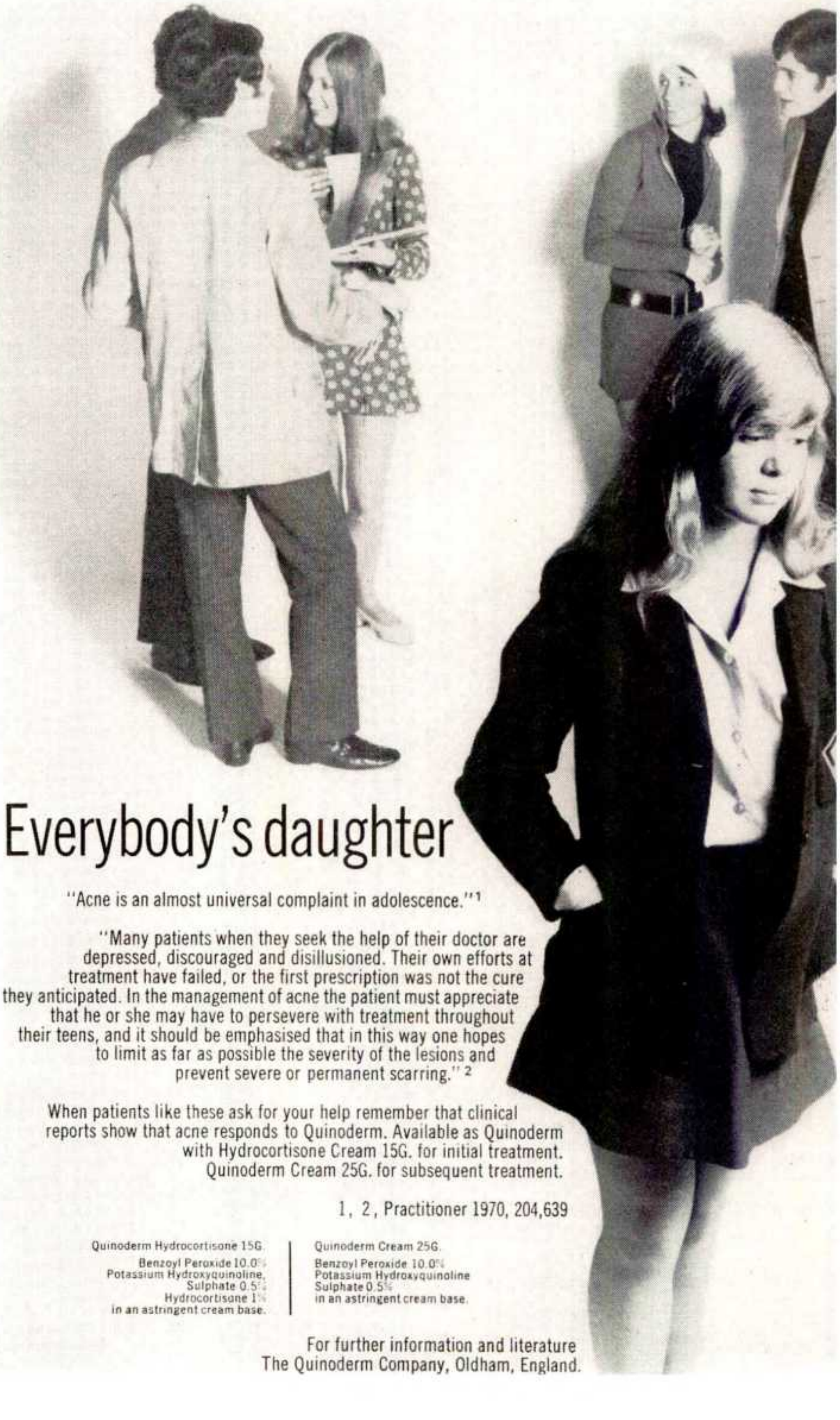

Everybody's daughter

"Acne is an almost universal complaint in adolescence."[1]

"Many patients when they seek the help of their doctor are depressed, discouraged and disillusioned. Their own efforts at treatment have failed, or the first prescription was not the cure they anticipated. In the management of acne the patient must appreciate that he or she may have to persevere with treatment throughout their teens, and it should be emphasised that in this way one hopes to limit as far as possible the severity of the lesions and prevent severe or permanent scarring."[2]

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1, 2, Practitioner 1970, 204,639

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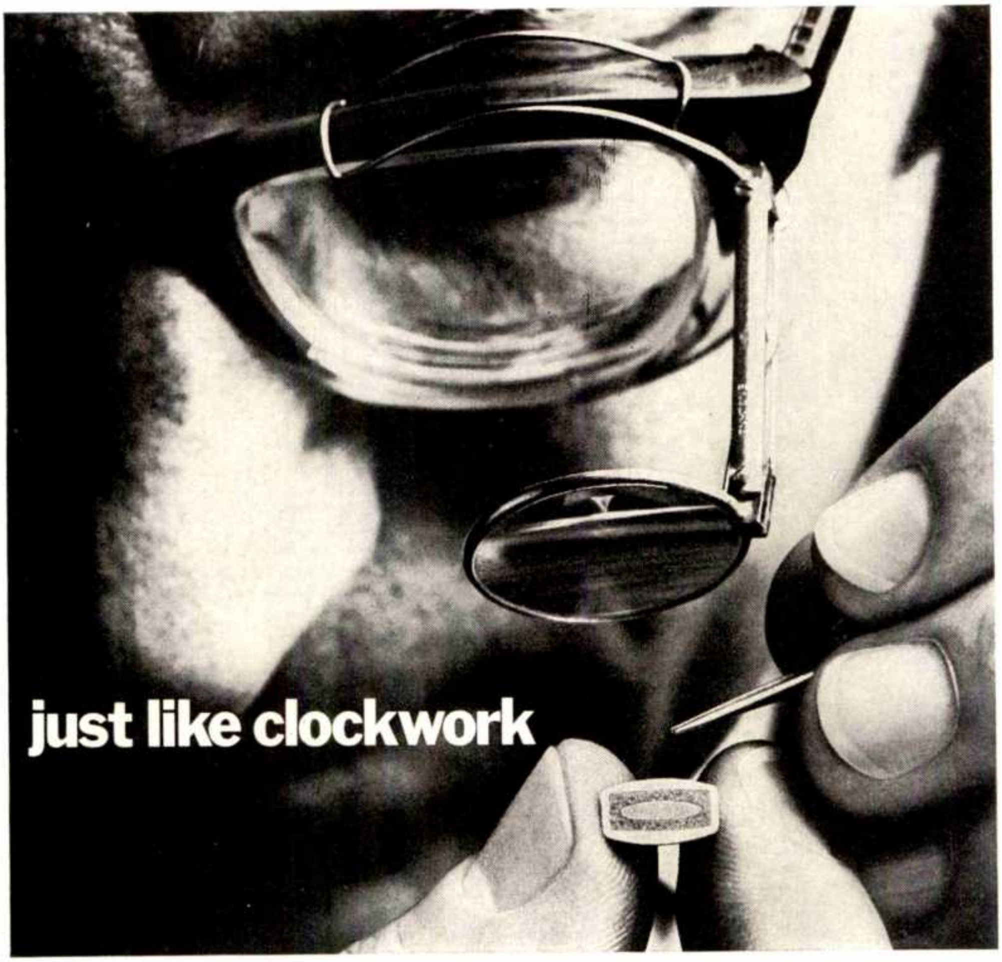

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CONTENTS (*continued*)

Obituaries
Reginald Thomas Brain 435
Archibald Gordon Smith 436

Correspondence
The Prevalence of Dermatitis Herpetiformis
D. Burrows 437
F. F. Hellier 438
Fixed Drug Eruption due to Phenazone in a Hypnotic
Julian Verbov 438
Zinc and Copper in Psoriasis
M. W. Greaves 439
Turkey Diet Regime in the Treatment of Psoriasis
Agnese Kelly 440
Treatment of Hyperhidrosis with Topical Propantheline—a New Technique
R. H. Seville and J. C. Frankland 441

Books
Sjögren's Syndrome (Shearn); Iatrogenic Diseases (D'Arcy and Griffin); Colours for Cosmetics. 3rd Report of the Farbstoff-Komission of the Deutsche Forschungsgemeinschaft; Epidermolysis Bullosa (Gedde-Dahl); The Significance of Physical Signs in Medicine (Mills) . . 442

Quarterly Review
Contact Dermatitis XVIII 445

News and Notices 447

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

Clinical and Laboratory Investigations

PAGE

Suppressed Cell-Mediated Immunity in Two Adults with Atopic Dermatitis
WALTER C. LOBITZ, Jr., JUAN F. HONEYMAN and NORMAN W. WINKLER 317

"Lymphomatoid" Pityriasis Lichenoides: a Variant with Histological Features Simulating a Lymphoma. A Clinical and Histopathological Study of 15 Cases with Details of Long Term Follow Up
MARTIN M. BLACK and EDWARD WILSON JONES 329

Ultrastructural Aspects of Dermatitis Herpetiformis (Duhring's Disease)
F. GUERRA RODRIGO 348

Variations in Skin Surface Lipid Composition and Sebum Excretion Rate with Different Sampling Techniques II
J. A. COTTERILL, W. J. CUNLIFFE and B. WILLIAMSON 356

Physico-Chemical Factors Influencing Epidermal Damage by Surface Active Agents
A. B. G. LANDSDOWN and P. GRASSO 361

Localized Unilateral Hyperhidrosis—a Clinical and Laboratory Study
W. J. CUNLIFFE, C. E. JOHNSON and D. M. WILLIAMSON 374

Progressive Changes in Cuticular Pattern along the Shafts of Human Hair as Seen by Scanning Electron Microscopy
EVA BOTTOMS, EDWARD WYATT and STANLEY COMAISH 379

Intracellular Localization of Tyrosinase Inhibitor in Amelanotic and Melanotic Malignant Melanoma
TOSHIO HAMADA and YUTAKA MISHIMA 385

Ultrastructural Differences in Human Malignant Melanomata. An Electron Microscopical Study
HERBERT KLUG and WOLFGANG GÜNTHER 393

Pharmacology and Treatment

Combined Cytostatic and Surgical Treatment of Extensive Basal Cell Epitheliomata
GERHARD WEBER and WILHELM MEINERT 408

Case Reports

Extraction of Pemphigus Antibodies from a Lymphoid Neoplasm and its Possible Relationship to Pemphigus Vulgaris
N. K. SAIKIA 411

Coiffure Keloids
MARGARET A. H. BAYLES 415

Comment

Precancer and the Skin
P. C. H. NEWBOLD 417

(*Continued on* p. iii *of cover*)

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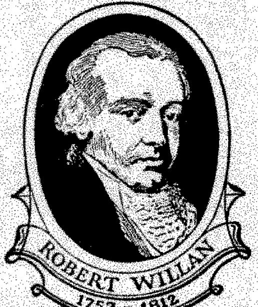

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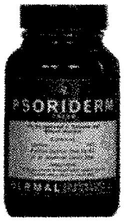

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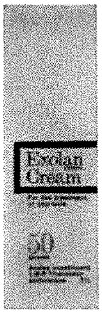

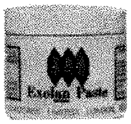

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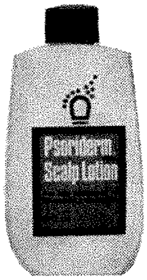

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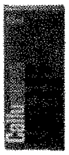

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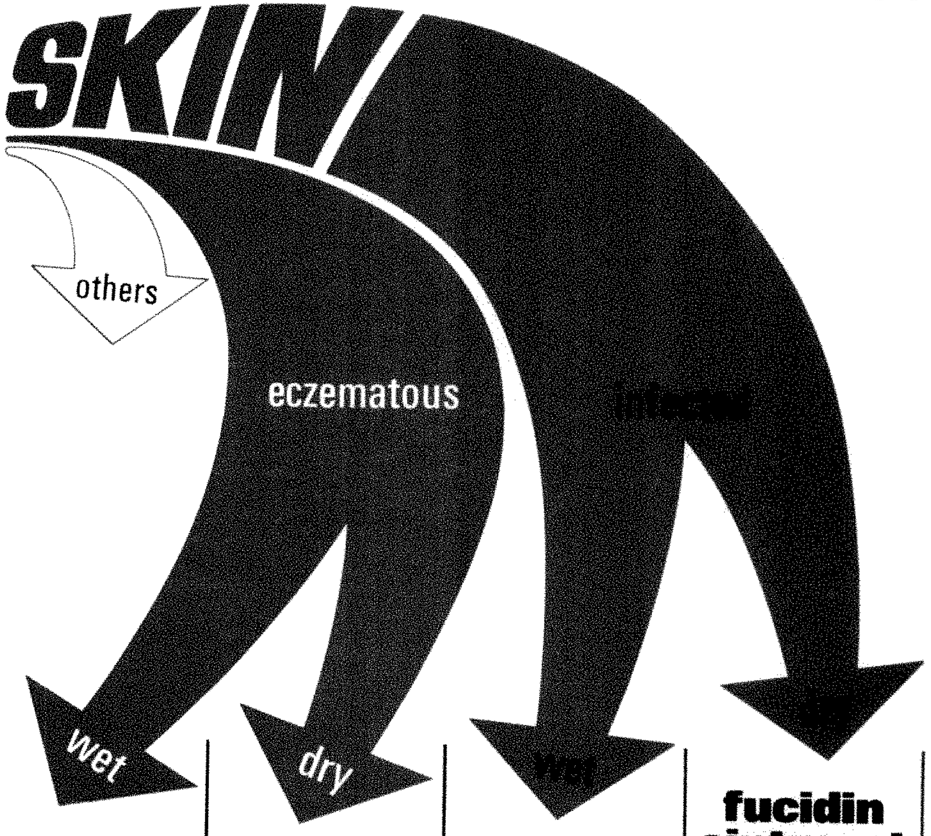

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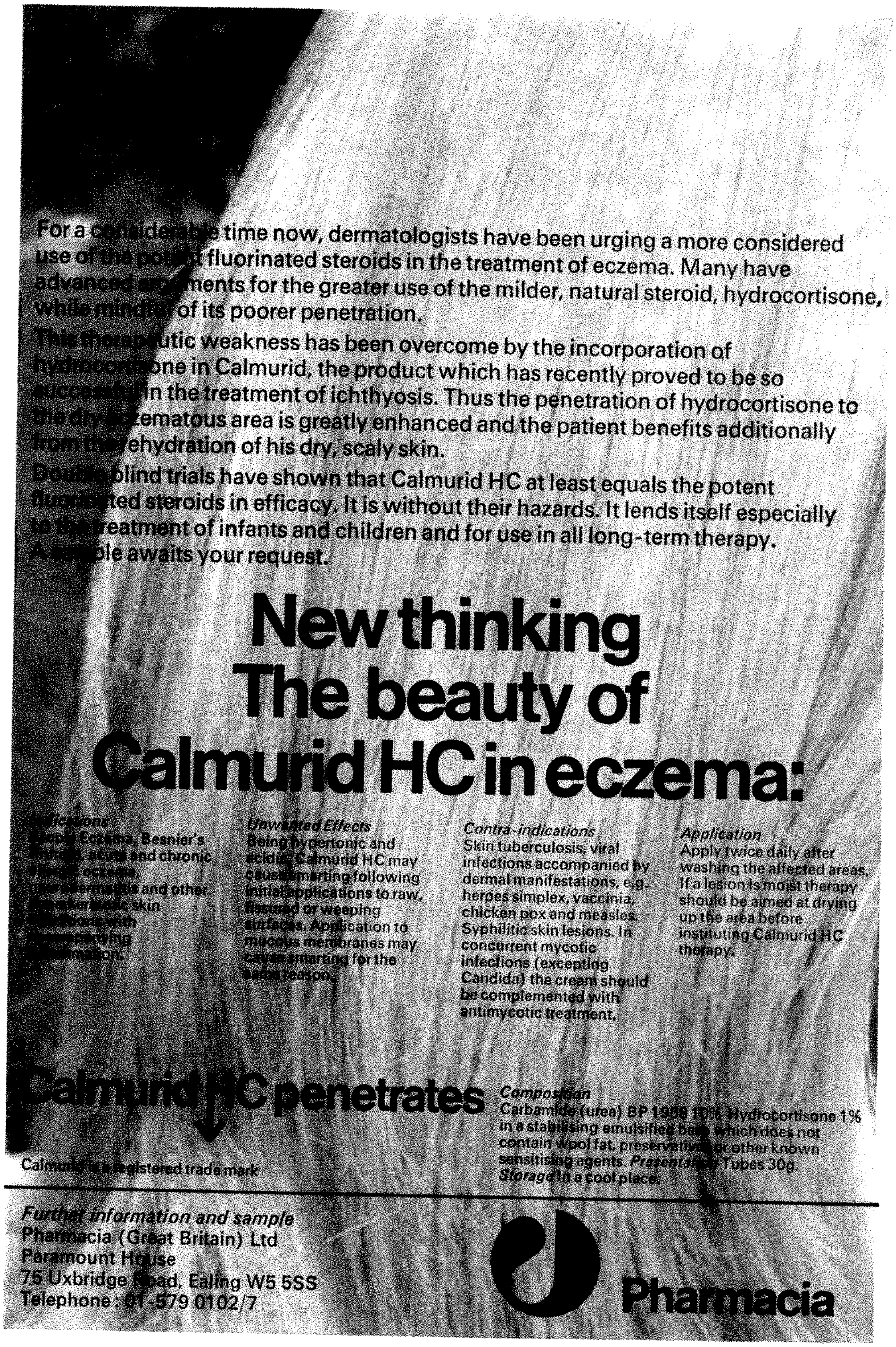

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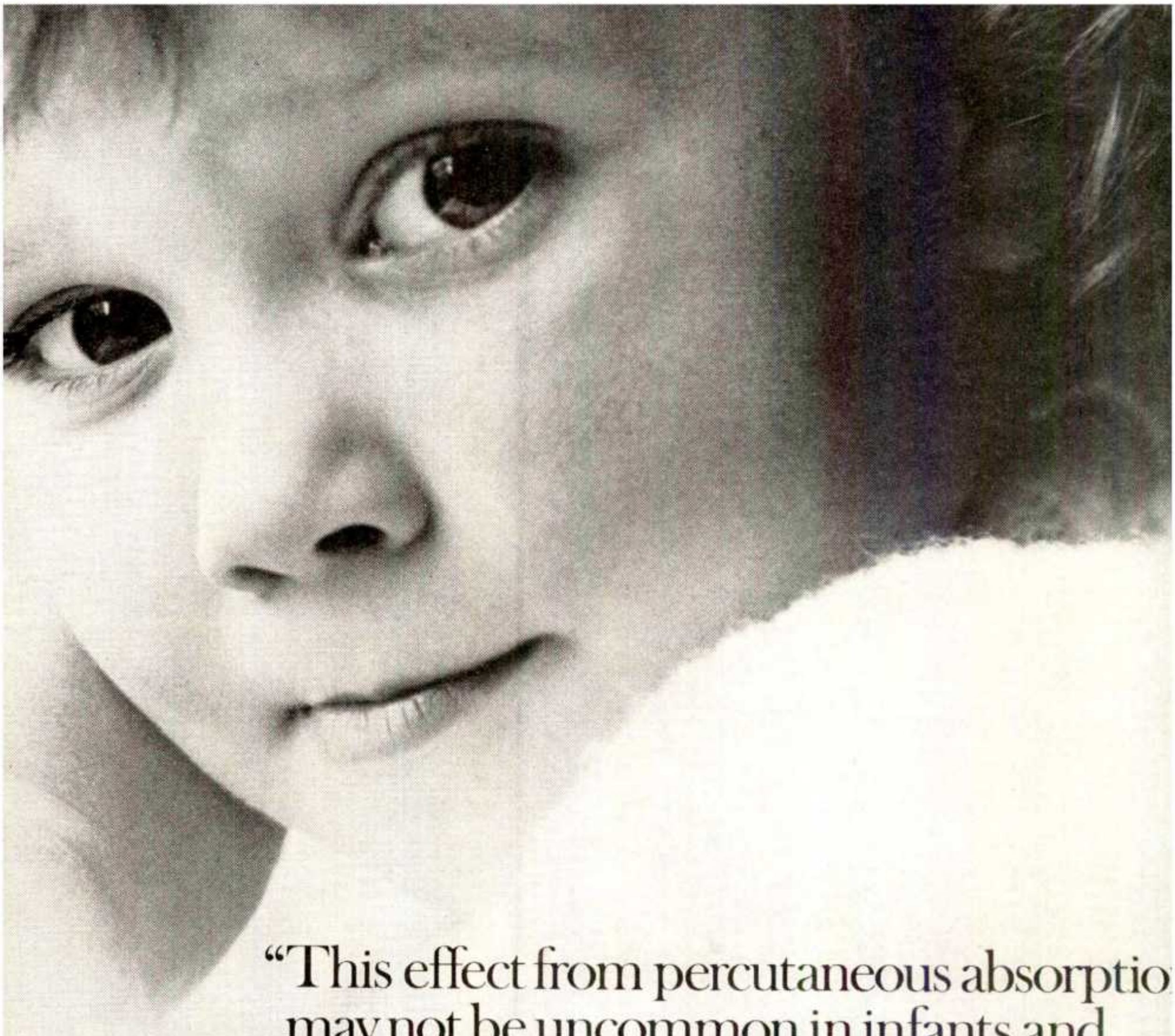

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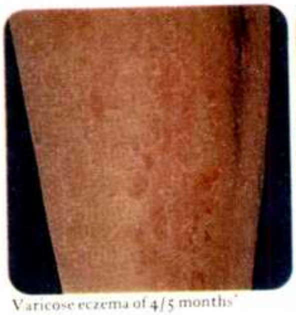

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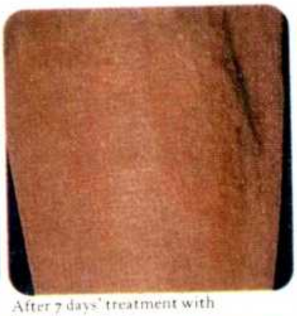

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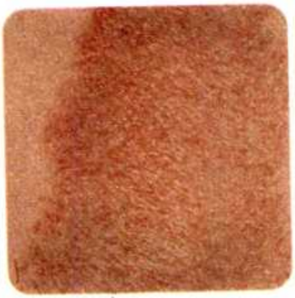

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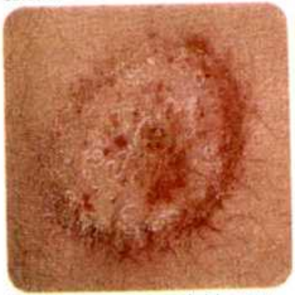

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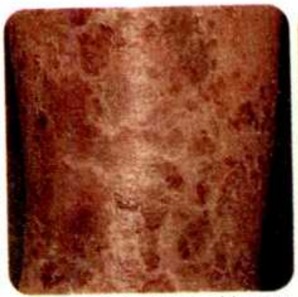

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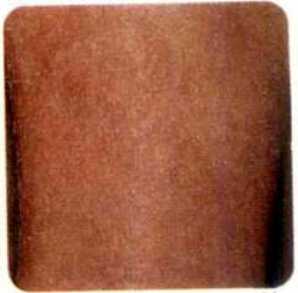

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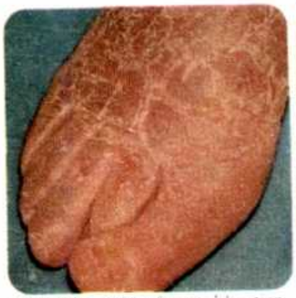

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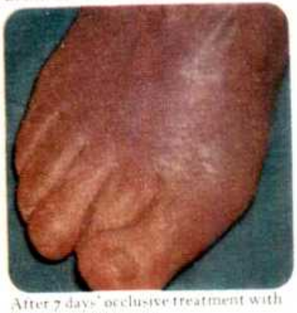

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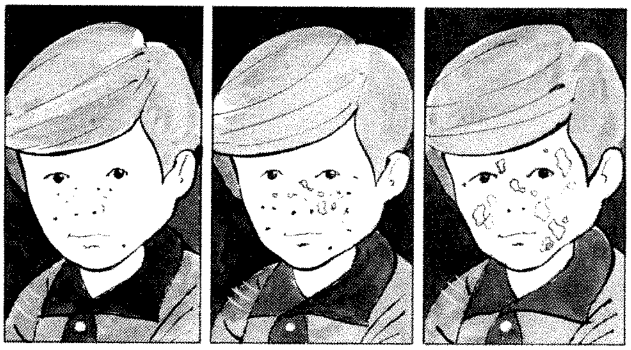

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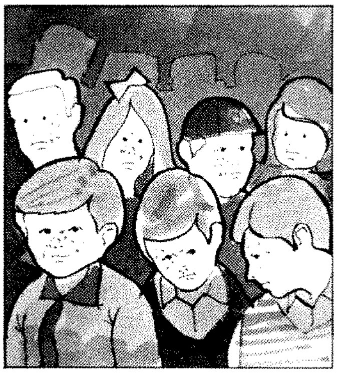

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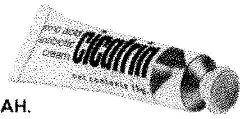

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TETRACYCLINE-RESISTANT β-HAEMOLYTIC STREPTOCOCCI IN DERMATOLOGICAL PATIENTS

JOHN-ERIK BRORSON, GÖSTA ROUPE AND STAFFAN SEEBERG

Summary.—During a 2-month period 221 isolates of β-haemolytic streptococci were cultured from patients from different clinics in Gothenburg and environs; 20% of the isolated strains were resistant to tetracycline. Among the different clinics, the Department of Dermatology showed the highest frequency of resistant strains (47%). In this clinic the majority of the patients with resistant strains were suffering from secondarily infected chronic dermatoses and had frequently attended the hospital. The treatment of streptococcal skin infection is briefly discussed.

The occurrence of β-haemolytic streptococci resistant to tetracycline was first reported by Lowbury and Cason in 1954. Since then there have been several reports on this subject (Kuharic *et al.*, 1960; Parker *et al.*, 1964; Robertson, 1965; Mitchell and Baber, 1965; Cybulska, 1970). The resistant strains have been isolated from various infections, especially of the respiratory tract, but there have so far been no reports based on dermatological material.

In a study from London, Dadswell (1967) reported that the frequency of resistant strains isolated mainly from the ear, nose and throat increased from 0·4% in 1958 to 44% in 1965. In a recent follow-up study from the same centre a decline in tetracycline-resistant strains to about 27% has been observed (Rees, 1971). The question as to whether this decrease was due to a more restricted use of tetracycline was not commented upon by the author.

MATERIAL AND METHODS

During a 2-month period (February–March 1971) all strains of β-haemolytic streptococci producing soluble haemolysin as judged by tube-tests were registered as regards clinical source and sensitivity to tetracycline.

The strains were isolated on horse-blood agar plates incubated aerobically at 37°C and read after 24 and 48 h. To detect strains with decreased susceptibility to tetracycline antibiotic sensitivity tests were performed according to Ericsson (1960). All strains with a zone of inhibition of 24 mm or less around a disc containing 50 μg tetracycline were investigated further. Material from a loopful of an 18-h culture grown in brain-heart infusion-broth was applied to blood agar plates containing tetracycline chloride in dilutions from 0·5 μg/ml to 128 μg/ml. These plates were checked for growth after 24 h. The MIC (minimum inhibitory concentration) value was defined

Accepted for publication December 5th, 1971.

as the lowest concentration completely inhibiting growth. Strains capable of growing on plates containing 1 μg/ml tetracycline or more were considered to be resistant.

The classification of all tetracycline-resistant strains and of 50 unselected tetracycline-sensitive strains into Lancefield's groups A, C and G was based on the acid extraction method of Lancefield (1928). Strains not possible to classify in this way were extracted by hot formamide according to Fuller (1938). The serological reactivity of the extracts was tested in micro-immuno-diffusion plates as described by Wadsworth (1962).

In all, 221 isolations of β-haemolytic streptococci from 221 patients in Gothenburg and environs were investigated.

RESULTS

Table I shows the minimum inhibitory concentration of tetracycline for the 221 isolated streptococcal strains. Forty-five strains with a decreased susceptibility to tetracycline had MIC-values between 2 μg/ml and 64 μg/ml. The majority of these strains (78%) had MIC values of 16 μg/ml or more.

TABLE I.—*Minimum Inhibitory Concentrations (μg tetracycline/ml) for 221 Strains of β-haemolytic Streptococci*

MIC (μg/ml)	No. of strains
≦ 1	176
2	1
4	4
8	5
16	3
32	24
64	8
	221

Serological grouping of the 45 tetracycline-resistant strains employing specific antisera against the Lancefield groups A, C and G showed that 39 (87%) belonged to group A and 4 (9%) to group C. Two (4%) were not classifiable (i.e. did not belong to Lancefield's groups A, C or G). One group A strain and 2 group C strains were classifiable only with Fuller's method. Two group C strains and the 2 non-classifiable strains were isolated from the Department of Dermatology. Grouping of 50 unselected tetracycline-sensitive strains showed that 43 (86%) belonged to group A and 3 (6%) to group C. Four (8%) were not classifiable. Thus there was no difference between resistant and sensitive strains as regards distribution within Lancefield's groups.

The distribution of strains from different clinical sources is seen in Table II. In all, 20% of the investigated streptococcal strains were resistant to tetracycline. Of streptococcal strains isolated from other sources than the Department of Dermatology 15%, 12% and 12%, respectively (mean 13%), were found to be tetracycline-resistant.

TABLE II.—*Number of β-haemolytic Streptococci Isolated from Different Sources*

	Tetracycline sensitive	Tetracycline resistant	Total	Percentage resistant
Respiratory tract	56	10	66	15
Skin infections				
Department of Dermatology	25	22	47	47
Other clinics	80	11	91	12
Miscellaneous	15	2	17	12
	176	45	221	20

The highest frequency of resistant strains (47%) was found in patients from the Department of Dermatology, Gothenburg. The resistant strains were isolated mainly from patients with secondarily-infected chronic dermatoses such as leg ulcers, atopic dermatitis and ecthyma of the leg, and these patients had frequently been admitted to the hospital (Table III).

TABLE III.—*Distribution of Tetracycline-Resistant and Tetracycline-sensitive β-Haemolytic Streptococci in 2 Main Groups of Patients from the Department of Dermatology*

	Tetracycline-sensitive streptococci	Tetracycline-resistant streptococci
Chronic dermatosis with secondary infection	10	20
Short-term infections in patients with otherwise normal skin	15	2
	25	22

Six patients of the 22 with resistant strains had been treated with tetracycline during the last few weeks. Some of the remaining patients with resistant strains had received tetracycline some months before the investigation, while others had not received tetracycline. Only 1 of 25 patients with sensitive strains had been treated with tetracycline during the same period.

DISCUSSION

Sporadic cases of tetracycline-resistant strains of haemolytic streptococci were isolated during the period from different clinics in Gothenburg and environs. In contrast to the moderate figures from other sources, the high frequency (47%) of resistant strains from the Department of Dermatology in Gothenburg is striking. The reasons for this could be the result of (a) emergence of resistant strains as a result of local and/or systemic administration of tetracycline or (b) hospital transmission of resistant strains.

Patients with venous leg ulcers and pyoderma often have mixed infections, which has been an indication for topical and/or systemic treatment with tetracycline. Recent tetracycline therapy could not be established for more than approximately 25% of the patients with resistant strains. On the other hand, the majority of resistant streptococcal strains were isolated from patients with frequent contacts with the hospital. These facts suggest that a combination of the 2 factors mentioned above may be responsible.

It should be kept in mind that patients with venous ulcers of the leg often have impaired circulation in the lower leg, and the possibility that oral treatment might give subeffective tetracycline levels in the ulcers could not be excluded. Streptococci exposed *in vitro* to increasing concentrations of tetracycline have been shown to develop resistance (Lowbury and Hurst, 1956).

There is clinical evidence that local treatment alone can result in emergence of resistant strains. Tetracycline-resistant β-haemolytic streptococci were found in 6 of 8 patients with leg ulcers during a 2-month period in the autumn of 1970; the treatment in these cases had been topical not systemic tetracycline (Perdrup, 1971).

All wounds in the skin harbour bacteria, and in most cases antibiotic treatment should not be instituted unless there are clinical signs of infection. The presence of β-haemolytic streptococci may constitute an exception to this rule, considering the risk of streptococcal nephritis. When antibiotic treatment in a case of streptococcal skin infection is considered, systemic penicillin therapy should be used (Wannamaker, 1970). The problem of anti-bacterial treatment in mixed infections must be considered individually. If systemic antibiotic treatment is considered in an ulcer with the very common combination of *Staphylococcus aureus* and β-haemolytic streptococci, penicillin is suggested as the drug of choice. Penicillinase-producing *Staphylococcus aureus* may require a penicillinase-stable penicillin, although benzathine penicillin G and penicillin V have been found to be effective in healing streptococcal skin infections in children, even if penicillinase-producing staphylococci were isolated from the same wound (Dillon, 1970). So far, no β-haemolytic streptococci with decreased susceptibility to penicillin have been isolated from clinical material.

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THE ORAL CONTRACEPTIVE AND VARIEGATE PORPHYRIA

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Summary.—The effect of the oral contraceptive on previously asymptomatic variegate porphyria is described. The importance of the presence of X-porphyrins in variegate porphyria is discussed.

THE appearance of cutaneous porphyria in men with prostatic carcinoma who were treated with oestrogen (Walsh, 1963; Becker, 1965; Vail, 1967) suggested that oestrogens contained in the contraceptive pill might affect adversely those individuals with pre-existing abnormalities of porphyrin metabolism. Degos *et al.* (1969) reported the occurrence of porphyria cutanea tarda after the prolonged ingestion of oral contraceptives. The much rarer variegate form of porphyria may be exacerbated or made manifest by oral contraceptives, and we now report such an instance in a previously asymptomatic woman to add to the 2 previously documented cases (Dean, 1965; Baxter, 1967).

CASE REPORT

A married woman (and trainee nurse) aged 20 was admitted to a surgical ward of the Norfolk and Norwich Hospital in July 1970 with a one-week history of abdominal pain, low backache, complete constipation and vomiting. She was afebrile, dehydrated and showed generalized lower abdominal tenderness. The sclera were mildly icteric. The rectum was empty, but the sigmoid colon was easily palpable and seemed to contain a large amount of faeces. Straight x-ray of the abdomen was normal, and no cause for her symptoms was suggested by the gynaecological and orthopaedic opinions which were sought. The abdominal pain gradually abated and her constipation was partially relieved by enemata given over the course of one week, at which time she was discharged home. Minovlar (Schering Chemicals Ltd.) had been started one week prior to the onset of her symptoms and had been discontinued at her admission on her own volition.

Obstinate constipation, abdominal pain, anorexia and weight loss continued through August 1970; and while on the same contraceptive for 7 days in late August, redness and swelling of the facial skin occurred after 30 min exposure to sunshine. Blisters and erosions gradually appeared around the nail folds and on the dorsa of the hands; and when Minovlar was taken again for 6 days in mid-September 1970, accentuation of abdominal pain, with constipation and vomiting, occurred, together with intense itching of the face and hands.

When admitted to the Skin Ward on September 23rd, 1970, she was afebrile, with normal pulse rate, respiration rate and blood pressure. There was evidence of marked weight loss, but no jaundice. Examination of the abdomen revealed diffuse tenderness in the lower half, but no other abnormality. Mentally she was well orientated and showed no neurological abnormality. The face, neck, dorsa of hands and palmar creases were pigmented. Superficial punctate scarring was present on the forehead

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and cheeks, with tense blisters and patchy denudation of skin on the dorsa of the hands and fingers (Fig. 1). The urine was dark brown in colour and, on the basis of a positive Watson-Schwartz test for porphobilinogenand the urinary and faecal porphyrin estimations (see Table I below), a diagnosis of variegate porphyria was made. Abdominal symptoms and signs abated spontaneously over the course of one week, but a tendency to blister formation following trauma and sun exposure persisted over the course of the next 4 months. Nine months after her presenting symptoms she was able to sunbathe without adverse effect, and the diffuse hyperpigmentation had faded.

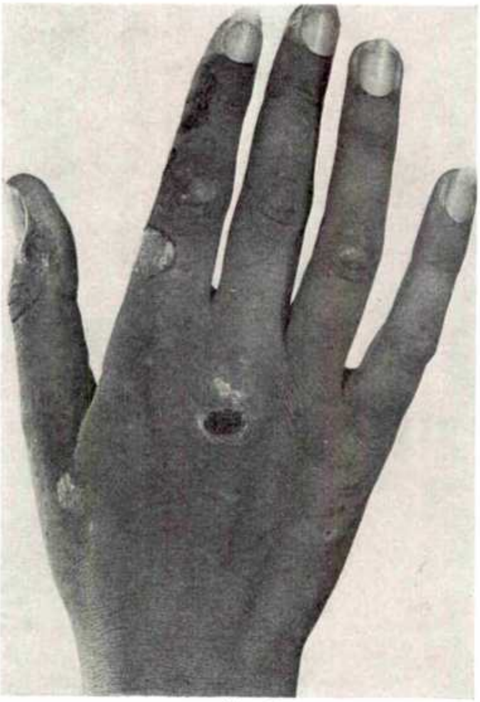

Fig 1.

Additional Laboratory Investigations

On first admission in July 1970, serum bilirubin was 2·0 mg% and S.G.P.T. = 33 R.F. units. These abnormalities returned to normal over the course of 2 months and thereafter liver function tests, together with the following haematological, serological and biochemical investigations, were in the normal range. Hb, W.B.C., E.S.R., L.E. cells, antinuclear test, W.R., V.D.R.L., Reiter's C.F.T., serum iron, serum iron binding capacity, B.S.P. excretion, blood urea, serum protein electrophoresis, serum electrolytes, blood sugar, urinary amino-acid chromatography, urinary 17-ketosteroids and 17-hydroxycorticosteroids.

TABLE 1.

Porphyrins	December 1970	July 1971	Normal values
Erythrocyte coproporphyrins	1·0 μg/100 ml cells	—	0–4·2 μg/100 ml cells
Erythrocyte protoporphyrins	16·0 μg/100 ml cells	—	0–37 μg/100 ml cells
Urinary coproporphyrins	962·0 μg/24 hours	846·0 μg/24 hours	1–283 μg/24 hours
Urinary uroporphyrins	159·9 μg/24 hours	128·7 μg/24 hours	0–41 μg/24 hours
Urinary δ-aminolaevulic acid	10·9 mg/24 hours	Nill detected	0–5·3 mg/24 hours
Urinary porphobilinogen	8·65 mg/24 hours	7·78 mg/24 hours	0–3·6 mg/24 hours
Faecal coproporphyrin	759·0 μg/g dry wt.	620·0 μg/g dry wt.	0–50 μg/g dry wt.
Faecal protoporphyrin	1482·4 μg/g dry wt.	1247·0 μg/g dry wt.	0–113 μg/g dry wt
Faecal X-porphyrin	437·0 μg/g dry wt.	640·7 μg/g dry wt.	0–20 μg/g dry wt.

Light testing.—July 1971 (Dr Ian Magnus).

At 300 nm an erythema after 90 seconds and erythematous papule after 300 seconds' exposure. M.E.D. at 137 mW-sec/cm^{-2}.

At 307·5 nm an erythema after 90 seconds' and erythematous papule after 300 seconds' exposure. M.E.D. at 175 mW-sec/cm^{-2}.

No response elicited after 300 seconds at 320 nm.

No response elicited after 900 seconds at 340 nm, 360 nm, 380 nm, 400 nm, 500 nm, 600 nm, and 700 nm.

These tests with the monochromator are within normal limits, and photosensitivity could not be demonstrated at the time of testing.

Biopsy of blister from hand.—This showed a subepidermal blister, with epidermal changes of spongiosis, moderate intracellular oedema and considerable hyperkeratosis. The dermis appeared normal.

X-ray, chest.—Normal heart and lung fields. Calcification of costal cartilages.

DISCUSSION

Variegate porphyria (VP) is so called because it may manifest symptoms due to photosensitivity and skin fragility, or it may occur with episodes of abdominal pain and neuropathy similar in symptomatology to the attacks of acute intermittent porphyria (AIP). In general, light sensitivity is usually found in the male members of an affected family, while the acute attacks occur more frequently in affected females.

VP is usually inherited as a Mendelian dominant character, and the sexes are equally affected. The parents of the young woman we describe are separated, and it has not been possible to screen the asymptomatic father or paternal relatives. The patient's mother and sister, neither of whom have symptoms, have a normal urinary and faecal pattern of porphyrin excretion. Dean (1971) has estimated that the disease occurs at the rate of 3/1000 in the white population of South Africa, and has traced this very high incidence of a dominant gene back to a marriage between Dutch settlers in the Cape in 1688. His study of the number and size of families arising from this union has shown that VP in no way impaired fecundity.

While AIP may show exacerbations during or after pregnancy in the absence of exogenous factors (Goldberg, 1959), pregnancy in VP usually passes without incident. Complications and the potential lethality of the gene became apparent only with the introduction of the barbiturates. Apart from the large reservoir of the disease in South Africa, the condition is much less common in other parts of the world, although accurate figures are not available.

It was fitting and perhaps not unexpected that Dean (1965) in South Africa should be the first to describe the adverse effect of oral contraceptives in a woman aged 20 whose VP had been heralded by abdominal pain and emotional symptoms after exposure to sulphonamides. His patient developed itching of the skin, with blisters and sores on the exposed parts, after taking an oral contraceptive (Lyndiol) for 2 weeks. Mental orientation was normal and abdominal symptoms were absent. Mild jaundice was present and was associated with abnormalities of liver function and the typical porphyrin excretion of the condition. Dean (1965) considered the clinical picture to differ from that seen in attacks of VP induced by barbiturates, where abdominal and neuropsychiatric symptoms are usual. The picture of photosensitivity and " hepatitis " which he described is in part mirrored by our own case, although her photosensitivity, initial jaundice and transient biochemical findings of liver derangement occurred on a background of severe abdominal pain. The case described by Baxter and Permowicz (1967) was clinically compatible with VP, and had exacerbations of photosensitivity and abdominal pain following the use of an oral contraceptive. Derangement of liver function was present initially, but no details of stool porphyrin were given.

The diagnosis of VP is usually self evident on the basis of symptomatology and a positive family history. Biochemically, a high level of faecal coproporphyrin and protoporphyrin are found in states of remission, and these levels usually remain high in acute episodes of the condition, when there are the added features of increased urinary coproporphyrin, porphobilinogen and δ-aminolaevulic acid. These findings are in contrast to the normal levels of faecal porphyrins which are found during remissions of AIP. There is disparity between increased faecal coproporphyrin and normal protoporphyrin during quiescent phases of hereditary coproporphyria with high urinary levels of coproporphyrin during remission and relapse. In some instances of porphyria cutanea tarda (PCT), where aetiological factors such as heredity and exogenous toxins are important, differentiation from VP with atypicality of clinical features and an unhelpful family history may be difficult. In these circumstances estimation of the so-called X-porphyrins may be helpful. These porphyrins have been recognized by several workers in the past, but were more fully characterized by Rimington *et al.* (1968) as porphyrin–peptide conjugates of varying amino acid content which occurred in the faeces and urine of individuals with VP. X-porphyrins are present in very small amounts in the faeces of normal individuals (0–20 μg/g dry wt.), but in very large amounts in VP. A moderate increase may be seen in AIP, but not to the degree seen in VP. Magnus (1971) has referred to the place of X-porphyrin assay in diagnostic chemical pathology. Not only does it help in the differentiation of VP from some similar cases of PCT, but its presence in a dominantly inherited condition makes genetic counselling easier.

Precise type definition of porphyria has important implications in the avoidance of drug-induced exacerbations. Magnus (1971) feels that there is no definite evidence for avoiding sulphonamides and barbiturates in PCT where the chief intolerance is to alcohol, oestrogens and chloroguin. In AIP and VP sulphonamides and barbiturates may precipitate acute episodes and should never be given. Goldberg (1971) has tabulated those drugs which have been reported to exacerbate porphyria, and in VP, in addition to sulphonamides and barbiturates, it would appear sensible to avoid dichloral-phenazone (Welldorm), pentazocine (Fortral), methyldopa, griseofulvin, chlorpromazine and oestrogens, including the contraceptive pill.

The enzyme δ-aminolaevulic acid synthetase (ALA synthetase) controls the rate limiting step in porphyrin synthesis from the basic glycine and succinyl-CoA. Dowdle *et al.* (1967) have demonstrated excessive hepatic ALA synthetase activity in VP, and they considered that this enhanced enzymatic activity was sufficient to explain the profile of porphyrin excretion in the condition. Excessive hepatic ALA synthetase activity, however, was found by the same authors in AIP and in symptomatic porphyria. This uniform and striking hepatic abnormality suggests that it cannot be the specific derangement responsible for acute clinical episodes. Since neither porphobilinogen or δ-aminolaevulic acid can induce the symptoms of an acute attack, it can only be assumed that these acute, non-cutaneous signs and symptoms are due to a disturbance of cellular or subcellular metabolism as yet undetermined.

Taddeini and Watson (1968) have suggested that in cutaneous porphyria a genetic abnormality may be present in hepatic cells, with possible diminution of the activity of drug metabolizing microsomal enzymes. The inability of such cells to dispose of substances such as alcohol and oestrogens could account for the induction of ALA synthetase and the subsequent over-production of porphyrins and their precursors through differing metabolic pathways. The precise mechanism of the skin damage is still undetermined, but investigations have indicated that photosensitivity results from porphyrins in the skin. Rimington *et al.* (1967) have summarized and further investigated the photobiology of porphyria and have reported that, in nearly all photosensitive patients with erythropoietic protoporphyria, PCT and VP skin reactions were found at the 400 nm region and to a lesser extent at the 500–600 nm band, a finding which corresponded well with the known absorption spectrum of porphyrins. They pointed out that in these 3 diseases photosensitivity was present to the same spectral band in spite of marked differences in their basic biochemistry. No photosensitivity could be detected by the monochromator in the case we describe. She was, however, tested at a time (July 1971) when she was in clinical remission in spite of persistent biochemical abnormalities.

Using the analogy of PCT induced by oestrogen, we have assumed that our patient's change from latent to symptomatic VP was induced by the oestrogenic component of her contraceptive pill. Perlroth *et al.* (1965) succeeded in diminishing the clinical features of AIP by the cyclical use of oral progestational agents. In the chick embryo, however, Rifkind *et al.* (1970) have demonstrated the ability of oral contraceptives to induce hepatic ALA synthetase. The degree of enzyme induction appeared to be determined by the progestational part of the mixtures. We have not felt justified in challenging our patient with the progestational component of the offending oral contraceptive.

We are indebted to Professor A. Goldberg and Dr I. A. Magnus for porphyrin estimations.

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ALIMENTARY *CANDIDA ALBICANS* AND NAPKIN RASHES

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Summary.—Specimens of faeces from 217 children with napkin rashes aged less than 3 months were examined for the presence of *Candida albicans* The 85 infants from whose faeces *C. albicans* was isolated were divided into 2 groups. The children in one group were treated with oral and local nystatin, and those in the other group were given an oral placebo and local nystatin. Further specimens of faeces were examined 2 weeks and 2 months after starting treatment.

The children who received oral nystatin were less likely than the others to have *C. albicans* in subsequent faecal specimens, and their napkin rashes were less likely to relapse; but no differences reached statistical significance. One-third of the children who received the oral placebo also lost the *C. albicans* from their faeces within 2 weeks, and it is suggested that host factors determine whether or not infection by *C. albicans* is transitory or is followed by a period of commensalism.

In a previous investigation into the role of *C. albicans* in napkin rashes (Dixon *et al.*, 1969) we were able to isolate the organism from 41% of napkin eruptions of various types. The clinical and mycological findings suggested that in many cases the yeast was playing a substantial part in the disease process, while in some it was probably present as a contaminant or as a relatively harmless commensal. We also isolated the organism from the alimentary tract of 44% of our cases, but we were unable to define the relationship between *C. albicans* in the gut and its isolation from napkin eruptions any further than that its presence in one site was likely to be associated with its presence in the other.

In the present investigation we planned to explore this relationship in greater depth by identifying a group of infants with napkin rashes who also had *C. albicans* in the faeces, and then attempting to eliminate the organism from the alimentary tracts of half the group with oral nystatin. As the presence of *C. albicans* in the faeces was likely to be associated with infection of the napkin rash, we also gave local nystatin to all children. Our intention was to compare the subsequent clinical and mycological progress of the children who received oral nystatin with those who did not.

PATIENTS AND METHODS

Between June 1969 and November 1970 each of Bristol's health visitors was asked to request the co-operation of the mother of each of the next 7 children aged less than 3 months with a napkin eruption whom she encountered in the course of her routine visits and clinics. When the consent of the mother had been obtained, the health visitor completed a short form which included information on the length of history of the napkin rash and an estimate of its severity. A mild rash was described as consisting of a few

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spots or ulcers, while a severe rash was one which covered most or all of the napkin area. Rashes which did not fit either of these descriptions were classified as moderate. The mother was then asked to send a specimen of the infant's faeces to the mycology laboratory, where standard procedures for the isolation and identification of *C. albicans* were carried out in the way described in our previous report (Dixon *et al.*, 1969).

If no *C. albicans* was isolated, the health visitor was informed of the negative result, but no further action was taken. Children from whose faeces *C. albicans* was isolated were allocated alternately to treatment and control groups, and the general practitioner was asked to consent to the child's inclusion in the study. The health visitor was then asked to give to each child's mother a letter of explanation and instruction, and a supply of nystatin ointment which was to be applied sparingly at each napkin change for 2 weeks. In addition, those in the treatment group were provided with 24 ml of nystatin suspension, and those in the control group were given 24 ml of a placebo suspension, with instructions in each case for 1 ml to be given orally 4 times daily for 6 days. The health visitors did not know which children had the active preparation and which the placebo. Each mother was also asked to supply a second specimen of the child's faeces 2 weeks after beginning treatment.

Two months after treatment was begun the health visitor was asked to visit each child once more to obtain information on the outcome of the napkin rash and on whether the prescribed treatment had been given, and also to ask for a third and final specimen of faeces.

RESULTS

During the 18 months of the study specimens of faeces from 217 children aged less than 3 months with napkin rashes were examined for the presence of *C. albicans*. The yeast was found in 85 (39%) of these specimens. There were no statistically significant differences between the frequencies with which the organism was found in children of different ages or with napkin rashes of different duration, but *C. albicans* was significantly ($P < 0{\cdot}05$) more likely to be found in the faeces of children with severe rather than with mild napkin eruptions (Table I).

TABLE I.—*Comparison of Infants from Whose Faeces* C. albicans *Was and Was Not Isolated*

	C. albicans isolated from faeces	*C. albicans* not isolated from faeces
Total in group	85	132
Mean age (weeks)	5·0	5·4
Mean duration of rash (weeks)	2·0	2·2
Severity (%)		
Mild	25	39
Moderate	52	49
Severe	23	12

Of the 85 children with *C. albicans* in the faeces, 43 were allocated to the treatment group and 42 to the control group. The 2 groups were comparable in respect of age distribution, duration of napkin rash and severity of the eruption. In a number of cases second or third specimens of faeces were not submitted, and in 3 cases the cultures were obscured by bacterial overgrowth. Table II shows the results of mycological examination of those specimens which were received about 2 weeks and 2 months after starting treatment. Comparison of the 2 groups shows that a slightly greater proportion of those in the treatment group appeared

TABLE II.—*Results of Mycological Examination of Second and Third Specimens of Faeces, 2 Weeks and 2 Months after Starting Treatment*

	Total in group	Second specimen		Third specimen	
		C. albicans not isolated	*C. albicans* isolated	*C. albicans* not isolated	*C. albicans* isolated
Treatment group	43	21	19	20	16
Control group	42	14	19	14	14

to have lost their faecal *C. albicans* after 2 weeks and also after 2 months, but the differences are not statistically significant.

There were 25 infants in the treated group and 24 in the control group who were said to have received all the prescribed therapy and for whom all mycology

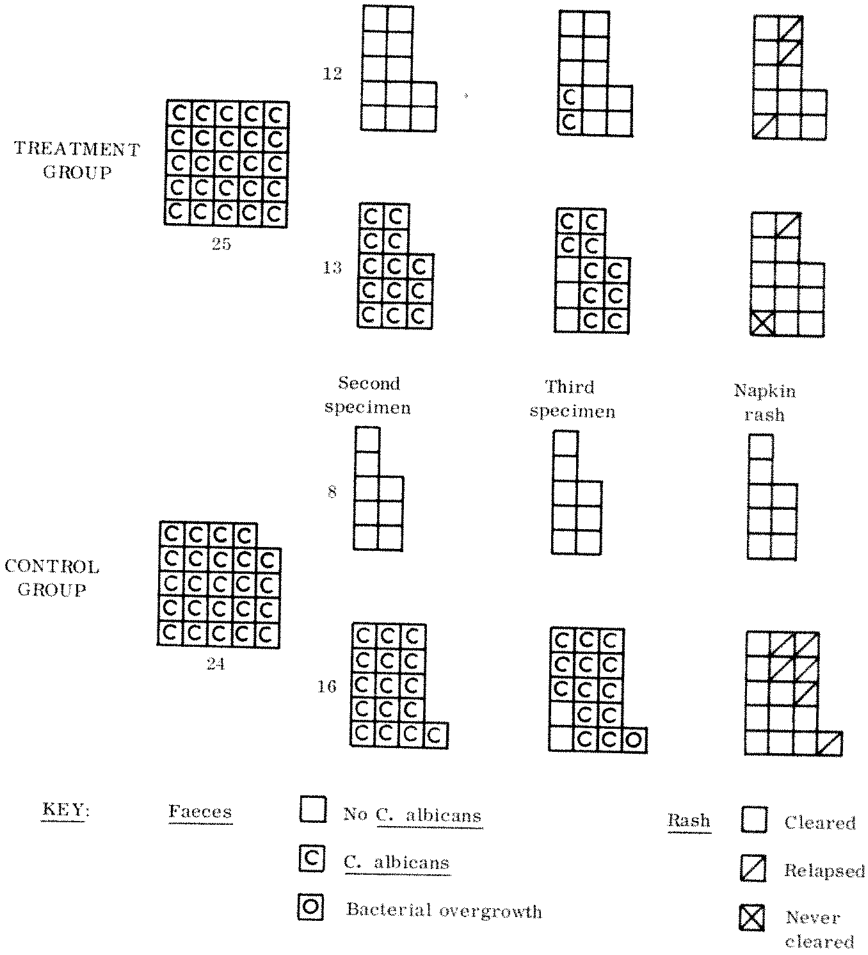

Fig. 1.—Results of subsequent examinations of faeces and the outcome of napkin rashes in children who were said to have received all treatment and for whom all results were available. Each ☐ represents one child.

results were available. The results given in Table III show no significant differences in the outcome of the napkin rashes in the 2 groups. In both groups the majority of the eruptions cleared and did not relapse. There was a tendency for more of the eruptions of those in the treatment group to clear without subsequent relapse; but, when clearing of the rash occurred in the control group, it tended to do so more quickly.

TABLE III.—*Outcome of Napkin Rashes in Children Who were Said to Have Received all Treatment and for Whom all Results were Available*

	Treatment group	Control group
Rash		
Cleared, no relapse	20	16
Cleared but relapsed	4	8
Never cleared	1	0
Time to clearing		
1–6 days	7	11
7–13 days	8	6
14 days or over	9	7
Never cleared	1	0
Total in group	25	24

The results of subsequent mycological examination of specimens of faeces and the outcome of the napkin rashes in those infants who were said to have received the prescribed treatment are shown diagrammatically in Fig. 1. The numbers are small and the cases are by now to a considerable extent self-selected, so only cautious interpretation is possible. In both groups some infants lost the *C. albicans* from their faeces and some retained it. There is a suggestion that those infants in the control group who lost the fungus within 2 weeks did well, in that the gut was not recolonized and the napkin rash did not recur. If *C. albicans* was isolated from the faeces of children in this group after 2 weeks, it was likely to be still there after 2 months, and there was a tendency for napkin eruptions to recur during this period. Infants in the treatment group were a little more likely to be free of faecal *C. albicans* after 2 weeks than those in the control group, but there was a tendency in a small number of cases for reinfection with *C. albicans* or relapse of the napkin rash, or both.

DISCUSSION

The results of this investigation raise more questions than they answer. If the study is looked on as a clinical trial of oral nystatin in the treatment of napkin eruptions which are associated with *C. albicans* in the alimentary tract, the results are inconclusive. The prescribed dose of oral nystatin frequently failed to eliminate the yeast from the bowel, but more of the treated than the control children lost their *C. albicans*, and their napkin eruptions were a little less likely to relapse. There is a need for further study on a larger number of infants with more effective methods of follow-up, and perhaps longer periods of treatment and certain precautions against re-infection.

The results in the control group are perhaps of particular interest. Of the 24 infants for whom all results were available, 8 lost their alimentary *C. albicans* within 2 weeks, and 2 more within 2 months. In none of these 10 infants did the napkin rash recur. In contrast, of the 13 infants in the control group who were known to have kept their *C. albicans* over 2 months, 5 suffered a napkin rash relapse. It is thus probable that the normal population includes some infants whose gut and skin are relatively resistant to colonization by *C. albicans*, and, if infection occurs, it is likely to be transitory; and also some infants whose intestinal environment is particularly favourable for *C. albicans*, which may live in the gut for long periods and periodically contribute to the development or persistence of a napkin rash. Treatment with oral nystatin may dislodge the organism in a few of these susceptible infants, but re-infection may occur.

This supposition is in keeping with the principle that infection with a micro-organism depends upon the relationship between the resistance of the host and the virulence of the agent. As far as *C. albicans* is concerned, the transition from commensalism to parasitism is probably determined not by changes in the virulence of the organism but by one of a number of possible physiological or pathological changes in the host (Winner, 1969). It is likely that host factors also determine whether infection with *C. albicans* is transitory or is followed by a prolonged period of commensalism. One can only speculate on what these factors may be, but further epidemiological or physiological investigations may be worth while. If some simple environmental factor such as diet is involved, then its identification and elimination may increase the effectiveness of antifungal therapy.

We are grateful to Messrs E. R. Squibb and Sons, Ltd, for supplying the nystatin and placebo preparations and for financial assistance; to Miss Linda Grenfell, for carrying out the bulk of the laboratory work; and to Bristol's health visitors, who made the study possible.

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A MICRO METHOD FOR MEASURING THE CARBON DIOXIDE RELEASE BY SMALL SKIN AREAS

F. A. J. THIELE AND L. H. J. VAN KEMPEN

Summary.—In search for rapid routine methods for the measurement of carbon dioxide release by human skin, the authors succeeded in improving the infra-red analytical method which, when carried out at 4·3 μm, yielded reliable results. Two techniques have been compared, viz. the steady state method (for larger skin areas of about 8 cm^2) and the trap technique (which is less dependent on the area to be measured: 1 to 10 cm^2).

With both techniques micro amounts of carbon dioxide, eliminated by very small areas of the skin and/or the sweat glands, can be determined: 2 to 100 parts/10^6 of CO_2 10 cm^{-2} min^{-1}.

It is known that human skin releases very small amounts of carbon dioxide (Amman and Galvin, 1968; Fitzgerald, 1957; Rothman, 1957; Schaefer and Scheer, 1951; Schulze, 1943; Schutter, 1968; Shaw *et al.*, 1929; Shaw and Messer, 1930). Several authors have attempted to discuss its significance (Ernstene and Volk, 1932a; Shaw *et al.*, 1929; Shaw and Messer, 1930). The alkali neutralization capacity of the skin has been related to this CO_2 release (Rothman, 1957; Schulze, 1943; Schutter, 1968), and experimental evidence has been presented to show that alkaline solutions stimulate the amino-acid and CO_2 release by the skin, perhaps by the sweat glands (Thiele and van Kempen, 1971). The wide variation in the values for CO_2 release, dependent on the site under investigation, might indicate that physiological variables are involved (Adamczyk *et al.*, 1966).

However, the pharmacological stimulation of the peripheral circulation (histamine) and sweat glands (pilocarpine) revealed no effect on the CO_2 release (Ernstene and Volk, 1932c). Besides this, anomalous CO_2 release appeared to occur at various stages of skin diseases (Ernstene and Volk, 1932b; Pototsky and Dyachenko, 1967; Pototsky and Scherbakov, 1968) and in older subjects (Ernstene and Volk, 1932a) who are supposed to show a decrease in sweat gland activity. A systematic investigation of CO_2 release, with measurements of sensible and insensible water loss (Thiele and van Senden, 1966; Thiele and van Senden, 1967; Thiele and Malten, to be published), seemed to be desirable.

For dermatological investigations it is essential that these CO_2 release measurements can be carried out on small skin surfaces. Therefore the method of measuring CO_2 spectrophotometrically by infra-red analysis at a wavelength of 4·3 μm was selected (Amman and Galvin, 1968; Ortega *et al.*, 1966; Karler and Woodbury, 1962; Schaefer and Scheer, 1951; Severinghaus, 1960). This communication presents a new approach to measuring CO_2 rapidly in micro amounts (parts/10^6/

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10 cm^{-2} min^{-1}) by using a trap technique yielding reliable results. This technique is suitable for routine determinations in skin physiological and dermatological research.

METHODS

Apparatus

1. *Infra-red carbon dioxide analyser URAS 2*, operating by absorption of radiated energy in the infra-red spectrum at a wavelength of 4·3 μm (specific for CO_2). This analyser is supplied with:

A cuvette with a pathlength of 250 mm: measuring range 0·0 to 0·01 vol % CO_2 (0 to 100 parts/10^6 of CO_2);
A thermostat;
A calibration curve, and (on request) with
An interference filter to suppress effects of water vapour (e.g. insensible and sensible perspiration and varying atmospheric water vapour pressures).

2. *Perspex skin cups*, covering skin surfaces of 1·8, 6·0, 8·0 or 10·0 cm², and provided with a gas inlet and outlet, and a metal ring with two slits for Velcro strips to attach the cup to the skin. To make sure that there is no leakage of atmospheric CO_2 into the measuring device, the cup can be closed by attachment to a large rubber stopper.

3. *Skin cup closing system*, consisting of 2 magnetic valves (Skinner) each provided with a gas inlet and outlet, connected to the inlets and outlets of the skin cup, the carrier gas supplier and the URAS meter. The Skinner valves are operated by a chronometer.

4. *Electronic second clock* (the chronometer), with a metric scale (Jaquet, type no. 308 BR), was provided with a start and stop switch. Thus the skin cup can be closed start) and opened (stop) electrically, thereby making it possible to trap the CO_2 for exactly measured time intervals.

5. *Carrier gas suppliers (A) and (B)*: CO_2 absorber Godart/Statham no. 12211 filled with Sodasorb.

6. *Galvanometer*, with a mirror scale for accurately reading the zero adjustment and full scale deflection at 100 parts/10^6 carbon dioxide (1 scale division per parts/10^6).

7. *Two flowmeters* (Rotameter no. 16441): maximum flow 500 ml air min^{-1} for additional control of the carrier gas flow and control of the flowrate of the nitrogen gas, flushing the URAS detector housing continuously, thus excluding interference from atmospheric CO_2.

8. *Viton tubes*: CO_2 impermeable; inner diameter 4 mm, outer diameter 8 mm.

9. *Strip chart recorder* 10 mV, with a disc integrator and printer (Vitatron).

10. *Gas mixing pump* Wösthoff, Model M. 200/a, for calibration of the URAS 2 with mixtures of nitrogen/air (with 0·03% CO_2).

11. *Nitrogen gas and liquid air cylinders*, provided with valves, and PVC tubings, inner diameter 4 mm, outer diameter 8 mm.

For simultaneous recording temperatures, use was made of:

12. *Yellow spring thermistors* for measurements of skin, carrier gas and ambient temperature. Two small banjo thermistors, mounted on adjustable springs, are attached to the inner wall of the skin cup, thus ensuring a constant contact between thermistor and skin surface. Two large banjo thermistors for measuring skin temperatures next to the cup. One air probe thermistor, attached inside the centre of the cup. One air probe thermistor for ambient temperature control.

13. *Thermistor measuring bridge* (6-channel), converting the temperature measurements to electric measurements which are subsequently registered on a Philips (Model PR) 6-channel recorder. The bridge is calibrated in such a way that the skin temperature

can easily be read on the recorder chart; by means of a switch 1 of the 6 temperature registrations can be selected.

14. *Water loss meter*, type Gow Mac Gasmaster no. 20–100, for measuring sensible perspiration with a ventilated skin cup, covering an area of 8 cm^2.

Set-up and Calibration

The whole assembly is given schematically in Fig. 1. To prevent spoiling of the magnetic valves by dust particles of the Sodasorb in the carrier gas suppliers, the outlets of these suppliers are covered with filter paper. Care must be taken that the valves are not contaminated with teflon particles from the teflon plates mounted in the holder for air-tight closure of the small skin cup (1·8 cm^2). For zero CO_2 setting and for moving the cup to another skin site, the URAS is provided with a switch for flow of CO_2-free air via the second carrier gas supplier.

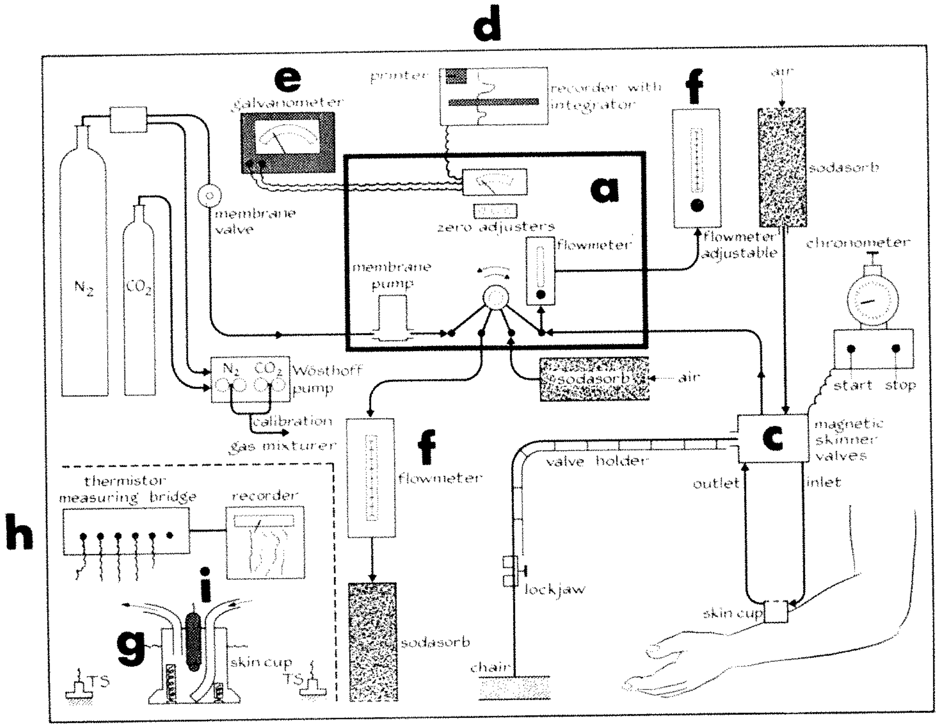

FIG. 1.—Schematic diagram of the assembly of the CO_2 loss measuring device with URAS 2 analyser (a), chronometer (b), magnetic valves (c), recorder (d), galvanometer (e), flow meters, (f), skin cup (g) and skin temperature measuring device (h), spring-mounted small banjo thermistors, inside the cup, and large banjo thermistors (TS) and air probe (i) thermistor.

The URAS meter can be calibrated by mixing known amounts of CO_2 (ex decompressed liquid air with 0·03 vol% CO_2) with dry nitrogen by means of the Wösthoff pump and measuring the extinction at 4·3 μm. The gas flow (100 ml min^{-1}) is additionally controlled by the Rotameter, connected with the gas outlet of the URAS meter. In this way, and with the aid of the calibration curve, provided by the manufacturer of the URAS 2 meter, its correct functioning can easily be checked.

Measuring Procedures

Two procedures have been developed which can be used alternatively, viz. the steady state method and the trap technique.

(a) *Steady state method*: The perspex cup is attached to the skin on the volar aspect of the forearm. A constant flow (100 ml min^{-1}) of carbon dioxide-free air is passsed through the cup. The carbon dioxide exuded by the skin is transferred by the carrier gas to the URAS analyser. From the deflection recorded the carbon dioxide concentration in the carrier gas can be calculated and the result is expressed in parts/10^6 CO_2/10 cm^{-2} min^{-1}; if the skin surface is 8 cm^2, the deflection must be multiplied by 1·25, etc.

(b) *Trap technique*: The difference from the steady state method is that, in the trap technique, the gas inlet and outlet of the cup are simultaneously closed for a limited and exactly recorded period of time. After exactly timed intervals of say 1, 3, 6 up to 15 min, the 2 magnetic valves are then opened again. The carbon dioxide released by the skin during these intervals is trapped in the skin cup and then transferred by the carrier gas to the analyser by means of a membrane pump mounted in the URAS meter. This can be done at flow rates of 100, 200 or 300 ml min^{-1}. This technique should be used if skin areas smaller than 8 cm^2 are to be measured. The total amount of carbon dioxide eliminated by the skin is recorded in less than 90 sec by the URAS meter, and the surface of the curve related to this amount is simultaneously integrated by the recorder and automatically printed in integration units. From the number of integration units per cm^2 (i.e. 6), the total surface area can be calculated. The carbon dioxide release is then calculated from the following equation:

$$CO_2 = \frac{a \times b \times c}{d \times e \times f} \times 100 \text{ parts}/10^6\ 10\ \text{cm}^{-2}\ \text{min}^{-1}$$

where

a = surface area recorded (cm^2)
b = calibrated recorder deflection for 1 part/10^6 of CO_2 (mm)
c = flow rate (ml min^{-1}) divided by 100
d = trap time (min)
e = chart speed (cm min^{-1})
f = measured skin surface (cm^2).

Thus, on measuring the CO_2 release on 8 cm^2 skin surface with a trap time of 3 min at a flow rate of 100 ml min^{-1}, the number of integration units recorded must be multiplied by a factor of 0·174 to obtain the CO_2 release in parts/10^6 10 cm^{-2} min^{-1}

$$\left(\frac{100}{3 \times 8 \times 4 \times 6} = 0{\cdot}174\right).$$

If the flow rate is 200 ml min^{-1}, this factor is 0·348. With a skin area of 1·8 cm^2 the factor is 0·772 at a flow rate of 200 and a trap time of 6 min.

For the conversion of parts/10^6 CO_2 10 cm^{-2} min^{-1} into ml $CO_2\text{ml}^{-1}1000\ \text{cm}^{-2}\text{h}^{-1}$, which is necessary for comparison with the values in the literature, the parts/10^6 values have to be multiplied by a factor of 0·6, as can be seen from the following example. The flow rate is 100 ml min^{-1}, which means 100×10^{-6} ml/mlmin^{-1} CO_2. The surface area should be multiplied by a factor of 100 and the time by a factor of 60. From this it follows that the factor is $100 \times 10^{-6} \times 100 \times 60 = 0{\cdot}6$.

RESULTS

(a) *Leakage*

Any leakage in the measuring system (URAS meter, tubing connection, carrier gas suppliers, etc.) can be determined by closing the measuring circuit during a trap time of 3 or 6 min and recording the number of integration units (integr. un.) after opening this circuit. Thus, the number of integr. un. recorded during a trap technique measurement on skin can be corrected easily: it is approximately 1 integr. un. min^{-1}.

(b) *Comparison of the Steady State Method and the Trap Technique on Normal Skin*

When the CO_2 measurements were carried out on normal skin successively by the steady state method and the trap technique, fairly equal results were obtained at equal skin temperatures, provided large cups covering a skin area of 6 or 8 cm^2 were used. The results are presented in Table I. At small skin areas of 1·8 or 1·0 cm^2, CO_2 measurements with the steady state method are impossible because the recorder deflections are of the same order of magnitude as the noise of the URAS meter on the recorder. This problem is not encountered in measurements with the trap technique (see Measuring Procedures b.); it supports the advantage of this technique.

TABLE I.—*CO_2 Release by Normal Skin*

CO_2 Release

(volar aspect of the forearm, measured skin surface 6 cm^2)

Subject	I Steady state method Parts/10^6	II Trap techniques values 10 cm^{-2} min^{-1}	Trap times (Min)	III Values obtained by multiplication of II by a factor of 0·6 ml 1000 cm^{-2} h^{-1}
No. 3	4·2	4·2	4	2·5
No. 4	4·2	4·0	5	2·4
No. 5	4·2	4·6	3	2·8
	4·8	5·3	5	3·2
No. 6	6·7	6·9	5	4·2
No. 5	14·8	14·4	3	8·7
	13·7	14·4	3	8·7

(c) *The Reliability of the Trap Technique Measurements*

1. *The flow rate of the carrier gas*: The reliability of the trap technique was demonstrated by carrying out CO_2 measurements at different flow rates (100, 200, 300 and 400 ml min^{-1} successively, without removing the cup from the measuring site on the forearm. The product of flow rate and number of integr. un. remained constant within experimental error (Fig. 2A, line a).

However, the recorder deflection, related to the steady state CO_2 transport through the skin, had to be adjusted to a constant value to overcome the lag period of the disc integrator of the recorder. It this lag period is present, a lower number of integr. un. are recorded (Fig. 2A, line b).

From the curve in Fig. 2 it can be seen that, at flow rates of 200 to 300 ml min^{-1}, minor variations in the flow will not markedly influence the measurement: here the curves are not too steep, whereas, between 100 to 150 ml min^{-1}, they are. In Fig. 2B the product of flow rate and number of integr. un. is plotted versus the flow rate: the straight horizontal line thus obtained confirmed these conclusions. Fluctuations in skin temperature, due to the flow of the carrier gas (cooling), were not observed.

2. *Disc and electronic integration*: The reliability of the area integration by the Vitatron recorder provided with a disc integrator and printer was controlled by

carrying out simultaneous area integration with an electronic integrator (Hewlett and Packard). One Vitatron integration unit at values of 27 and 30 integr. un., corresponded to 1363 and 1347 H & P integr. un., respectively. This relatively minor discrepancy between disc and electronic integration can be ruled out by an additive calibration with known amounts of CO_2.

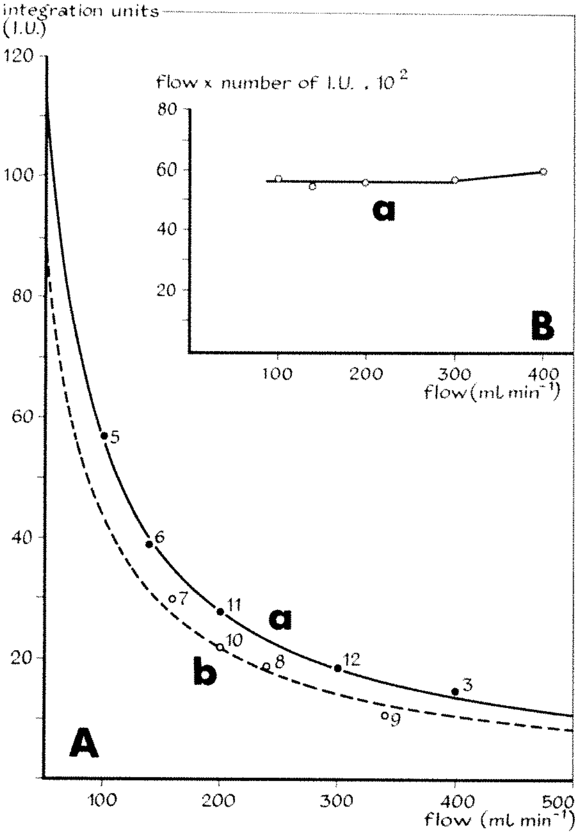

FIG. 2.—The flow rate of the carrier gas. A. Plot of the number of integration units (integr. un.) versus flow rate of the carrier gas (ml min^{-1}): the dotted line (b) presents the results without and the drawn line (a) with, adjustment of the recorder to a constant deflection. The numbers given on the curves indicate the sequence in which the continuously performed measurements were carried out. B. Plot of the product of the carrier gas flow rate (ml min^{-1}) and number of integr. un. versus flow rate: conversion of the (a) curve in 2A to the (a) curve in 2B.

3. *Water vapour interference, velocity of the CO_2 release and alternative calibration method*: The CO_2 measurements are not disturbed by small or large amounts of water vapour (insensible perspiration) trapped in the skin cup. The effect of the interference filter could be demonstrated by injecting small amounts of water (5 μl to 0·1 ml) in a perspex block with gas-chromatographic septum incorporated in the measuring device, near the skin cup. Different mixtures of nitrogen/air (0·03 vol% CO_2) were used. Moreover, by successively injecting known amounts of bicarbonate and lactic acid solutions in the perspex block via the septum, a quantitated minute amount of CO_2 will be released by their chemical reaction. The recorded amount of CO_2 can then be compared with the amount actually injected via the septum ($HCO_3^- \rightarrow CO_2$).

This is an alternative method for calibrating the URAS 2 meter and acts as a check on the correct functioning of the other registration devices (recorder, carrier gas suppliers, etc). Injection of small amounts of CO_2 saturated water in the Viton tube connections showed the complete release of CO_2 within 1 min, approximately.

4. *Trap time variation*: The reliability of the trap technique measurements with large skin cups (6·0 cm^2 surface) on a skin site on the forearm of an individual with a relatively high CO_2 release was demonstrated by the results with trap times of 1, 2, 3, 4 and 5 min; the CO_2 releases were found to be 8·8, 8·9, 8·5, 9·0 and 9·1 parts/10^6 10 cm^{-2}/min^{-1}, respectively. These measurements were carried

out successively, without removing the skin cup from the measuring site on the forearm. The CO_2 releases found with the steady state method were slightly higher: 10·0, 10·8, 10·8, 10·0 and 9·6 parts/10^6 10 cm^{-2} min^{-1} respectively, because the zero CO_2 gas calibration line had shifted below zero. This problem will not be encountered with the trap technique. The measurement with a small skin cup (1·8 cm^2 surface) on the forearm of a subject with a lower CO_2 release gave values of 3·6 and 3·7 parts/10^6 10 cm^{-2} min^{-1}, with trap times of 5 and 10 min respectively.

From these results it follows that, on increasing the trap times, and thus the CO_2 concentration in the skin cup, no reabsorption of CO_2 or decrease in CO_2 elimination occurs at different time intervals.

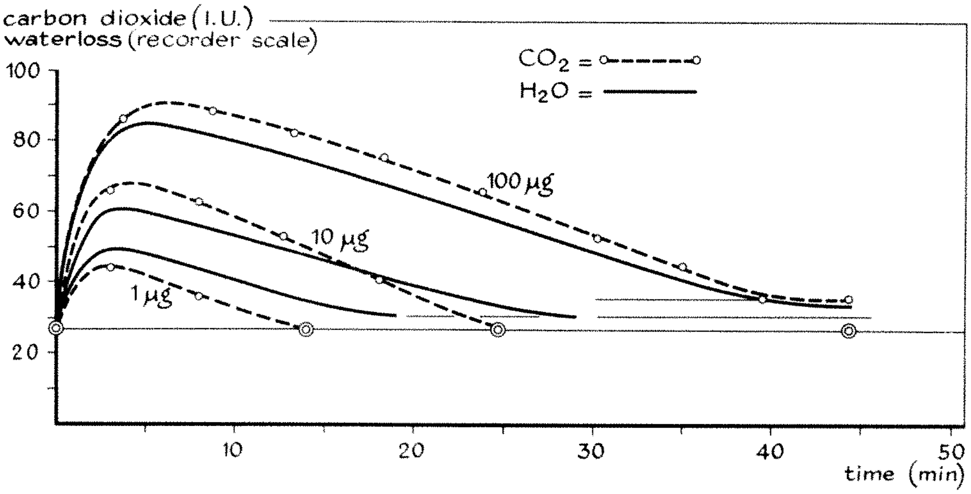

FIG. 3.—Carbon dioxide release and water loss curves (release versus time) after intradermal stimulation of the sweat glands with different amounts of acetylcholine (in 0·1 ml solvent).

5. *Skin cup size variation*: Measurements of the CO_2 release with small (1·8 cm^2/6 min) and large (8·0 cm^2/3 min) skin cups revealed values of: 8 integr. un. × 0·7716 = 6·17, and 18 integr. un. × 0·374 = 6·25 parts/10^6 of CO_2 10 cm^{-2} min^{-1}, respectively.

(d) *The Influence of Some Physiological Variables on CO_2 Loss*

1. *The relationship between skin temperature and CO_2 loss*: By varying the climatic conditions in the measuring room, the CO_2 losses decreased or increased with the skin temperature in a fashion similar to the variations found for the insensible water loss (Thiele and van Senden 1971).

2. *The effect of exposure to heat radiation on the CO_2 release by human skin*: The effect of exposure to heat radiation, although inducing very slight sweating, could be recorded on the skin of a normal subject; the initial values of 3·8 increase continuously to values of 4·6, 5·5, 7·5 and 14·4 parts/10^6/10 cm^{-2} min^{-1}, although the skin temperature remained constant. The increase occurred after exposure of head and neck of the subject to direct radiation by sunlight. Similar effects

were found with another subject whose CO_2 losses increased from 6·9 to 12·3 parts/10^6/10 cm^{-2} min^{-1}.

3. *The effect of acetylcholine stimulation on the CO_2 loss by skin*: Stimulation of the sweat glands by intradermal injections of acetylcholine (1, 10 and 50 μg/0·1 ml) and simultaneous continuous measurements of the CO_2 loss and H_2O loss (with the Gas Master) revealed fairly similarly shaped water loss and carbon dioxide loss curves, with similar disappearance-times (Fig. 3). These preliminary experiments indicate that large amounts of CO_2 can be exuded by the sweat glands, with the water. The method for writing a CO_2 loss curve, i.e. by using the results of intermittent trap technique measurements during a period of over 90 min, will be published shortly. Since the blank (initial) values of the CO_2 loss are measured before the stimulation, the increase in the CO_2 loss can be calculated in percentages.

DISCUSSION

From the results of our experiments it appeared that the CO_2 release by human skin could be measured quantitatively by infra-red analysis. The trap technique is less dependent on the surface area to be measured compared to the steady state method. It could be demonstrated that comparable results were obtained with both techniques. The CO_2 released from normal skin at different sites on the volar aspect of the forearm of different subjects varies widely: 3·5 to 15 parts/10^6/10 cm^{-2} min^{-1}. We should like to emphasize that the recent mass spectrometric evaluations of the CO_2 release by other skin sites of an apparently normal subject appeared to vary between 8·5 and 24·0 (breast), 18 (back of the trunk), 30 and 36 (cubital fossae), 42 (stomach), 42 and 48 (palms), 90 (forehead) and 325–425 (axillae) parts/10^6/10 cm^{-2} min^{-1} (recalculated values) (Adamczyk *et al.*, 1966).

The widely varying values might indicate that the sweat glands contribute to the CO_2 release (Schulze, 1943; Shaw and Messer, 1930); it has been reported that there is a sudden dramatic rise in CO_2 release at the onset of visible sweating (Rothman, 1957; Rothman and Schaaf, 1929; Shaw and Messer, 1939). Our results with exposures to heat radiation were very similar; the preliminary experiments with acetylcholine showed a striking relationship between water loss and CO_2 release. The increase in CO_2 loss at a higher skin temperature confirmed the findings of other authors; they reported that environmental temperature *should* be the principal factor influencing the rate of CO_2 elimination (and oxygen absorption) by the skin. The increased CO_2 release at higher skin temperatures may explain why the CO_2 losses increase with increasing cutaneous blood flow (Rothman, 1957), arterial hyperaemia (Kramer, 1928), inflammatory conditions (u.v. erythema) (Kramer, 1928; Rothman, 1957; Shaw and Messer, 1930) and decrease to minimum values at maximal vasoconstriction (Rothman, 1957; Rothman and Schaaf, 1929). Venous stasis, with greatly increased CO_2 content in the blood, however, does not increase the CO_2 loss (Ernstene and Volk, 1932a; Rothman, 1957; Schulze, 1943).

The results of the measurements carried out on normal and damaged skin with the CO_2 meter described in this communication will be published shortly.

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Instruments

1, 5, 6, 7, 8 and 10: Godart/Statham N.V., Bilthoven, The Netherlands.

9: Vitatron, Dieren, The Netherlands.

2, 3 and 13: These apparatuses were constructed at the Intrument Workshop of the University of Nijmegen (The Netherlands) by Mr J. G. van Wamel under the supervision of the Head of that department.

Schematic Circuit Diagrams are available on request.

12: Yellow Springs Instrument Co., Yellow Springs, Ohio (U.S.A.).

4: Jaquêt, Basel, Switzerland.

4: Stop and Start Switch Circuit Diagram is available on request.

14: Gasmaster No. 20–100, Gow Mac W10–454, Gow-Mac Instrument Co., Madison N.J. (U.S.A.).

13: Philips 6-channel Recorder PR. 3500/00 Philips—Eindhoven, The Netherlands.

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THE SELECTIVE DEPIGMENTING ACTION OF 8-HYDROXYQUINOLINE ON HAIR GROWTH IN THE MOUSE

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Summary.—Topical applications of 8-hydroxyquinoline to mouse skin cause depigmented hair to grow in patterns which change with time and appear to be closely associated with the hair growth cycles. Sufficiently frequent applications result in virtually complete depigmentation in young adult C57BL female mice, while single applications cause isolated bands of depigmented hair. Female mice are more sensitive than males, but some treated newborn mice were unaffected. 8-Hydroxyquinaldine has a similar action. Compounds not showing this effect include 2 recognized depigmenting agents, some compounds related to 8-hydroxyquinoline and some other analytical reagents for copper.

8-HYDROXYQUINOLINE (oxine; 8-quinolinol; 8-HQ) is a very versatile compound which has found a wide range of uses, particularly in analytical chemistry as a reagent for many metals and in pharmaceutical practice as an antibacterial, fungicidal and spermicidal agent. It has the distinction of a 4-volume work devoted to it and closely related substances (Hollingshead, 1954–1956).

Its important chemical characteristic is the ease with which it forms stable metal complexes involving both the nitrogen atom and the adjacent hydroxyl group, such as that formed between 2 molecules of 8-HQ and bivalent copper (Fig. 1).

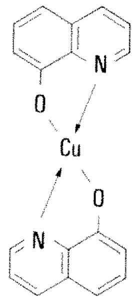

FIG. 1.

This is the basis of its analytical use. However, most other metal reagents tested do not possess the biological activity of 8-HQ, for which its fairly high stability constant and ease of penetrating cell walls are also important (Albert, 1968).

Because of its pharmaceutical applications it has been tested on a number of occasions for carcinogenic activity without giving very conclusive results. From the most recent and extensive tests, in which 8-HQ was administered to rats and mice by several routes, Pliss and Volfson (1970) concluded that 8-HQ is a weak carcinogen which may induce tumours indirectly by effecting changes in hormonal balance.

A quite unexpected action of 8-HQ, found during additional carcinogenicity experiments, has recently been described (Searle, 1970). Topical application at about 0·5% to the skin of mice of several pigmented strains was found to cause the growth of non-pigmented hair in striking patterns, which changed markedly with time and appeared to be dependent on the animals' hair growth cycles. The effect was most marked in C57BL mice, and females were considerably more sensitive than males. The present paper amplifies and extends these observations.

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EXPERIMENTAL

Compounds

8-Hydroxyquinoline (Koch-Light Laboratories Ltd., Colnbrook, Bucks.) was recrystallized from aqueous ethanol (m.p. 73–74°C) and 5-chloro-7-iodo-8-hydroxyquinoline from the same supplier from ethyl acetate (m.p. 177–178°C). Other chemicals tested were used without special purification, and were applied in Analar acetone unless otherwise stated.

Animals

Pure-line C57BL, IF and DBA mice, also C57BL × IF F_1 hybrids, were bred in this department, and CBA mice were supplied by the Department of Surgery. They were maintained on Thompson cube diet 42 and tap water *ad libitum*, and were ear-punched for identification.

Applications

The usual procedure employed was to clip the whole back area of each animal closely with an Oster small animal clipper with blade No. 80, size 40 (Brookwick, Ward and Co., London, W.6), then to treat the whole area twice weekly with 0·25 ml of the test solution in acetone applied from a graduated pipette. Variations from this are indicated in the text. Repeated clippings were frequently made, e.g. when investigating changes in pigmentation with time and in tests on compounds which showed no depigmenting action.

Histology

Dorsal skin was taken from some treated mice for histological examination so as to include adjacent pigmented and depigmented areas. The skin was fixed in 10% formaldehyde-saline solution. Blocks were cut at 6 μm and stained with Harris' H. and E., or by Schmorl's method for melanin.

RESULTS

Depigmentation by 8-Hydroxyquinoline

The depigmenting action of 8-HQ was first observed (Searle, 1970) during experiments in which the compound at 0·3% in acetone was applied twice weekly to the dorsal skin of C57BL × IF F_1 hybrid mice of both sexes. After 2 weeks of applications it was noticed that there was much white hair in the new hair growth of the female mice, but not of the males.

This unexpected effect was first followed up using groups of 10 male and 10 female C57BL, IF, C57BL × IF F_1 hybrid and C3H mice, also treated with 8-HQ but at 0·15% in acetone applied 4 times per week. After 7 weeks and several clippings, the only noticeable effect in the males was the presence of a few white hairs in the regrown coat. IF females showed very little depigmentation, while that in the C57BL females was noticeably more extensive than that in the hybrids. Considerable depigmentation occurred also in the C3H mice. There was marked variation between similarly treated mice of a given group, and no attempt has been made to quantify the effect.

As a result of these tests, most subsequent experiments with 8-HQ and other compounds were made, using female C57BL mice.

Strength of 8-HQ solutions.—Twice-weekly applications of 0·1% 8-HQ in acetone caused slight but definite depigmentation, but, at 0·3% and 0·5%, a wide range of striking patterns of normal and depigmented hair was obtained. Fig. 2 illustrates the marked variations in patterning between animals treated identically with 0·3% 8-HQ.

Increasing the strength to 1·0% did not significantly increase the degree of depigmentation. This strength had earlier proved toxic to some albino mice and has not been exceeded in these experiments.

Frequency of applications.—Increasing the frequency of 8-HQ applications to 5–6 per week did not markedly increase the degree of depigmentation. With 10–11 applications per week, however, some mice became very extensively depigmented over the treated area of skin (Fig. 3) and, contrary to the earlier impression, it now seems that complete depigmentation is obtainable by sufficiently intensive treatment.

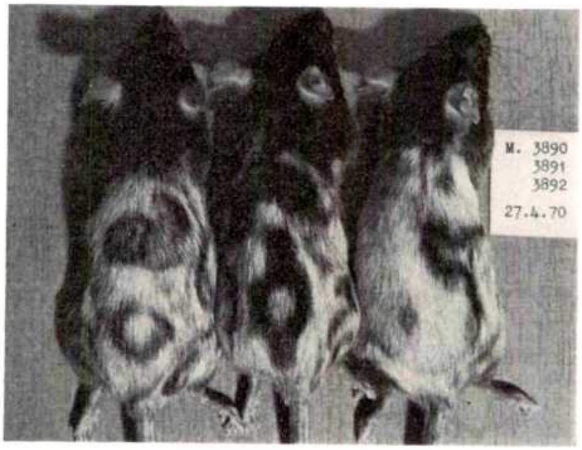

FIG. 2.—C57BL female mice treated with 8-HQ, 0·3% 4 times weekly.

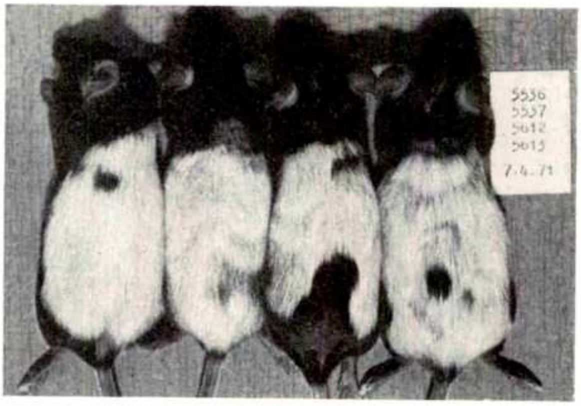

FIG. 3.—C57BL female mice; 8-HQ, 0·5% 11 times weekly.

The effect of single applications of 0·5% 8-HQ was also investigated in 16 young adult female C57BL mice. Isolated bands of depigmented hair grew on some mice as in Fig. 4, while others remained unaffected.

Changes in depigmentation patterns and the influence of age.—A number of C57BL mice were photographed at intervals during prolonged 8-HQ treatment. The photographs regularly showed marked changes in depigmentation patterns with time. The typical animal illustrated in Fig. 5 shows quite different patterning on 2 dates during 8-HQ treatment, while, 20 weeks after discontinuing treatment, the same animal had very little depigmented hair remaining. In such cases renewed treatment again results in depigmentation.

The many varied and striking patterns of depigmentation seen have occurred on mice treated from the age of approximately 3 months. Continued treatment in older mice (over one year) resulted in much more even distribution of depigmented hair and the disappearance of very distinct patterning. Contrary to expectation, a litter of 6 C57BL mice treated twice weekly with 8-HQ (0·5%, 0·1 ml) from the age of 4 days grew normal first coats of hair showing no sign of depigmentation.

Other methods of application.—Ten C57BL female mice aged 3 months were treated with 0·5% 8-HQ dissolved in water by the addition of a small amount of HCl (final pH 5–6). This solution could not be applied evenly from a pipette and was therefore rubbed into the skin on a little cotton-wool. This similarly caused patterns of depigmentation which changed with time. Application to 4 mice of 0·5% 8-HQ in liquid paraffin was also effective.

It was found that the above aqueous solution of 8-HQ could be given to mice as drinking water without evident adverse effect, but no depigmentation occurred in the 8 female C57BL mice treated. An isolated white spot on the back of one mouse was attributed to direct contact of the skin with the spout of the drinking bottle.

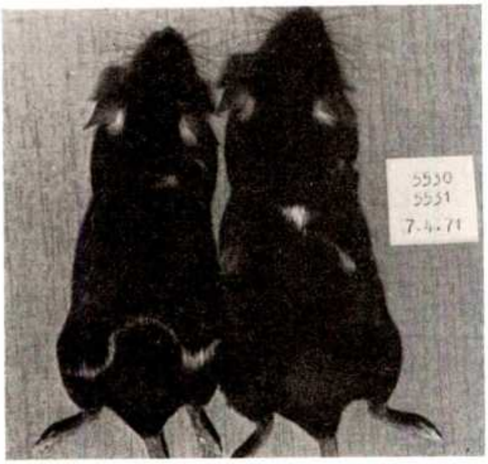

FIG. 4.—C57BL female mice; 8-HQ, 0·5% once only.

Other mouse strains.—Twice weekly applications of 0·5% 8-HQ in acetone to female IF mice caused quite distinct bands of depigmentation (Searle, 1970) although, as already mentioned, this strain is appreciably less susceptible than the C57BL. Other inbred strains showing considerable depigmentation were the C3H, CBA and the grey DBA (Fig. 6).

Other species.—While it would be of interest to test a wider range of species for 8-HQ depigmentation, this has only been done to date using 4 clipped areas on one black rabbit and on one black guinea-pig. No effect was seen in the rabbit and only a slight greying of hair on the guinea-pig.

Tests on Other Compounds

A number of other compounds have been tested for depigmenting action on groups of young adult C57BL female mice (Table I), some because of their structural relationship to 8-HQ, others being recognized analytical reagents for copper and other metals, or having known depigmenting action under different conditions.

8-Hydroxyquinaldine.—This is the only other compound found to cause depigmentation in mice in a similar manner to 8-HQ. As it differs from 8-HQ only in possessing a methyl group at position 2, its activity was not unexpected.

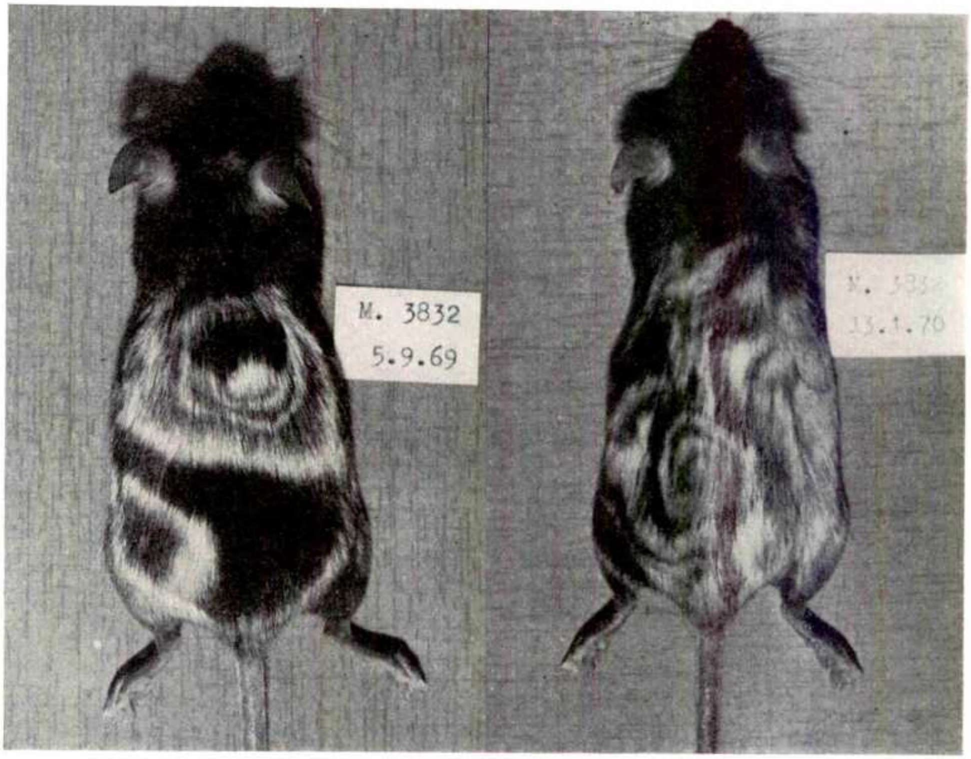

FIG. 5.—C57BL female mouse photographed at intervals; 8-HQ, 0·5% twice weekly.

TABLE I.—*Other Compounds Tested for Depigmenting Action by Twice-weekly Application in Acetone to the Skin of Young Adult Female C57BL Mice*

Compound	Strength (%)	No. of mice	Duration of experiment (weeks)
Active			
8-Hydroxyquinaldine	0·5	10	20
Inactive			
4-Hydroxyquinoline*	0·25	6	12
5-Hydroxyquinoline	0·5	6	12
5-Chloro-7-iodo-8-hydroxyquinoline (clioquinol)	0·3	15†	96
8-Mercaptoquinoline hydrochloride‡	0·5	6	11
2-Mercaptopyridine	0·5	6	12
α-Benzoin oxime (cupron)	0·5	6	12
Zinc diethyldithiocarbamate	0·5	5	10
2,2′-Biquinolyl	0·5	5	11
2,9-Dimethyl-1,10-phenanthroline (neocuproin)§	0·2	10	9
Dithio-oxamide (rubeanic acid)	0·5	5	30
2-Mercaptobenzthiazole	0·5	5	26
p-Benzyloxyphenol (monobenzone)	0·5	4	21
p-Hydroxypropiophenone (paroxypropione)	1·0	10	20

* Four times per week.
† C57BL × IF mice.
‡ In acetone/ethanol, 50/50 by vol.
§ Toxic at 0·5%.

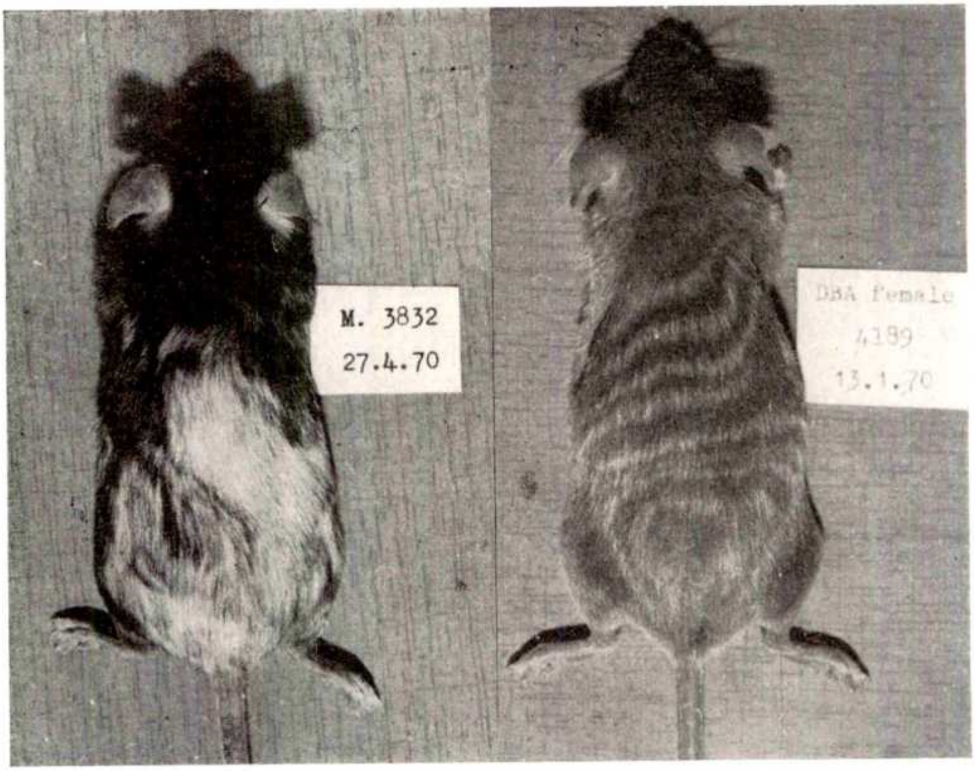

FIG. 6.—DBA female mouse; 8-HQ, 0·5% twice weekly.

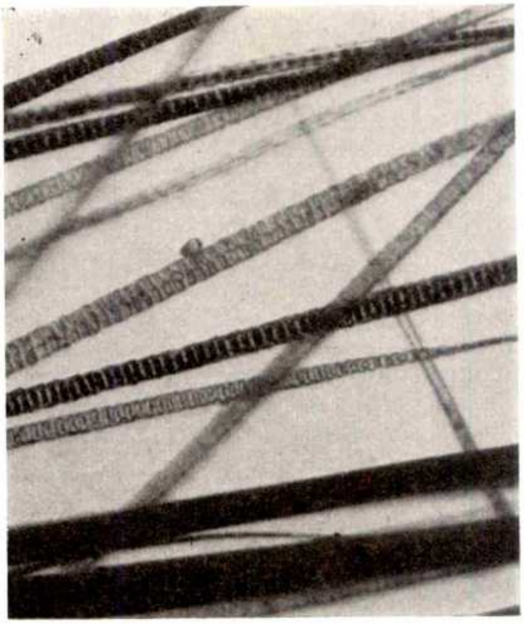

FIG. 7.—Clipped hair from C57BL mouse treated with 8-HQ, showing hairs with and without pigment. (× 140.)

Inactive compounds.—In the original experiment which showed up the depigmenting effect of 8-HQ, the drug 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol) was also tested on 15 male and 15 female C57BL × IF hybrid mice. Some were treated for as long as 96 weeks without showing any evidence of depigmentation, and this compound was not therefore tested further.

Twelve other compounds, tested by twice-weekly applications to young adult female C57BL mice for at least 9 weeks (Table I) similarly caused no depigmentation.

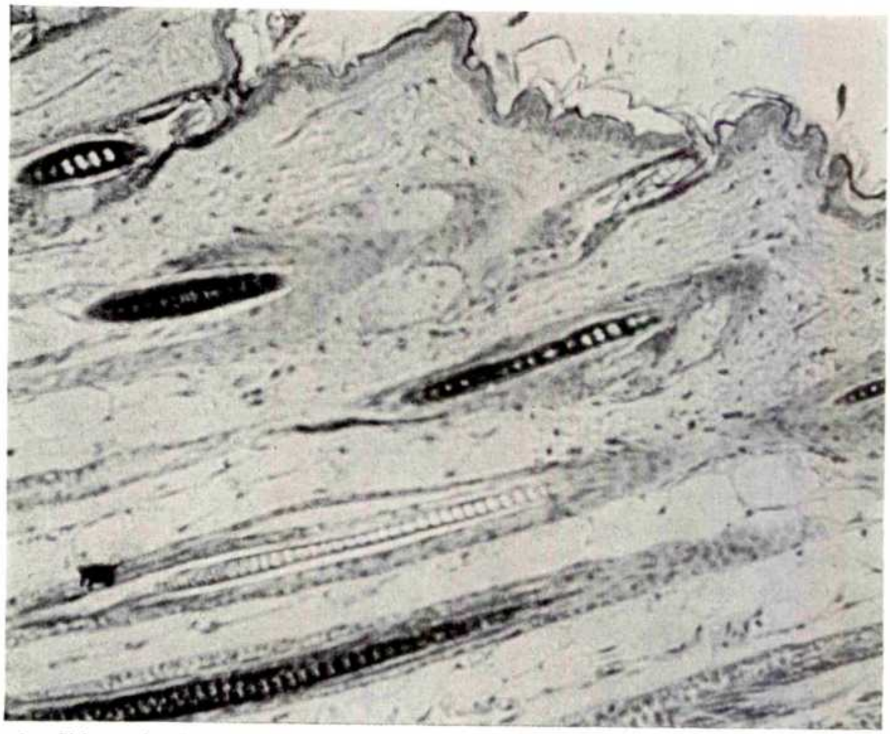

FIG. 8.—Skin section from C57BL mouse treated with 8-HQ, showing pigment-free hair in follicle with pigmented hairs in neighbouring follicles. (H. and E. × 140.)

Microscopy of Hair and Skin

Microscopic examination of hair clippings from 8-HQ-treated C57BL mice showed the presence of depigmented hairs, indistinguishable from those from albino mice, together with black hairs which appeared completely normal. The hairs were either completely white or completely black (Fig. 7). There was no banding, although a few hairs retaining some pigment at the tip were observed. The various types of hairs (monotrichs, etc.) were all seen among both pigmented and non-pigmented hairs.

Stained sections through treated skin which had grown both pigmented and non-pigmented hair were also examined. Suitable sections showed follicles containing non-pigmented hairs closely adjacent to others containing normal hairs (Fig. 8), but have not to date thrown any light on the mechanism responsible for the selective depigmentation effect.

DISCUSSION

Melanin is a complex material formed from tyrosine by a series of reactions involving the copper-containing enzyme tyrosinase (phenol oxidase), and there is a very considerable literature on the agents which inhibit its formation both *in vivo* and *in vitro*.

Injection of mice at 60 days of age with the carcinogen 3-methylcholanthrene besides inducing tumours, causes a variety of coat colour mutants which breed true (Strong, 1948), and the same agent fed to hamsters causes permanent but non-heritable depigmentation (Aubert and Bohuon, 1970). Other types of agent cause depigmentation which is reversible on cessation of treatment. Feeding of phenylthiourea, for example, causes reversible depigmentation on administration to black rats (Dieke, 1947), but has no effect in C57BL mice. p-Benzyloxyphenol and p-hydroxypropiophenone are 2 agents which produce skin depigmentation in man on topical application, but they were among the compounds found inactive in the experiments described here.

Although no record of 8-HQ as an inhibitor of melanogenesis *in vivo* has been found, it was one of a small group of compounds which produced over 90% inhibition of potato tyrosinase activity *in vitro* (Tsukamoto and Taniguchi, 1957). However, 3 of the other compounds (dithio-oxamide, 2-mercaptobenzthiazole and diethyldithiocarbamate) were all inactive in the present experiments, as were the other copper reagents: α-benzoin oxime, 2,2′-biquinolyl and 2,9-dimethyl-1,10-phenanthroline. This suggests that 8-HQ does not owe its depigmenting action to direct interference with copper-dependent enzyme action.

As the isomeric 4- and 5-hydroxyquinolines do not possess the important spatial relationship between the OH group and N atom present in 8-HQ, their inactivity was to be expected. However, the more closely related 5-chloro-7-iodo-8-hydroxyquinoline, 8-mercaptoquinoline and 2-mercaptopyridine were also inactive.

The depigmenting effect described in mice is thus restricted at present to 8-HQ and its methyl derivative 8-hydroxyquinaldine, but the mechanism of their action remains unknown. The antibacterial and antifungal actions of 8-HQ are, however, attributed to the toxicity of a metal complex formed by chelation *in vivo* (Albert, 1968), and it seems reasonable that such a toxic complex could also be responsible for the effects reported here.

Of perhaps greater interest than the actual occurrence of depigmentation with 8-HQ treatment is its selective nature, which results in the striking patterns of depigmentation described. No comparable effect appears to have been observed previously, although not dissimilar patterns of hair growth can be visualized in the albino rat by administration of the dye 9-phenyl-5,6-benzoisoalloxazine which becomes incorporated in the growing hair (Haddow *et al.*, 1945).

The coat of such mice consists of areas containing hairs which are entirely free of pigment, immediately adjoining other areas which, despite identical treatment, remain apparently quite unaffected. Hairs are not banded, as might have been expected if melanogenesis were inhibited in all follicles for a limited time after each application. Sections of treated skin (Fig. 8) show follicles in which melanogenesis is inhibited lying adjacent to others where it is apparently occurring normally.

The evidence available is thought to support the view that 8-HQ or a metal complex has a toxic action which prevents melanogenesis only at a certain critical stage early in the hair growth cycle. The effect is long-lasting, and follicles, once affected, continue to produce white hairs for the remainder of the cycle. Unless

8-HQ applications are made at least twice daily, other follicles can pass through the critical stage unaffected, and these then produce pigmented hairs throughout the cycle, despite repeated treatment.

On this view, the distinct single bands produced on some mice by a single application of 8-HQ (Fig. 4) show the positions of follicles passing through the critical stage at the time of application. The very varied patterns of bands and whorls produced by repeated applications then seem explicable in terms of the various types of hair growth in mice as worked out and illustrated by Borum (1954), while the replacement of distinct depigmentation patterns by a more even distribution in older mice also accords with the irregular overlapping growth cycles which then occur.

The depigmenting effect of 8-HQ described here is very simply produced and may provide a useful tool for research in the fields of melanogenesis and hair growth.

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THE GROWTH OF ADULT HUMAN SKIN *IN VITRO*

M. LEVINE

Summary.—In this report we investigate the role of serum in organ culture of adult human skin. The migratory overgrowth of dermis by epidermal cells is shown to depend on the presence of high molecular weight (mol. wt.) components of serum. Regeneration of the epidermis after 19 days' incubation occurs in the presence of 10% whole serum in the growth medium. DNA synthesis is found to increase in long-term incubations in medium including serum components but not in the presence of Minimal Essential Medium (MEM) only.

The traditional methods for organ culture of adult human skin have yielded reasonably consistent results to be useful for many experimental purposes (Reinertson, 1961; Reaven and Cox, 1965, 1968). However, there is a limited period during which epidermis can be maintained in culture, and within this period differences are observed from epidermis *in vivo*. The gross changes in the morphology of excised skin have been well documented at the light microscope level. Sarkany *et al.* (1965) described the 2 main distinctive alterations usually found, the migratory overgrowth of epidermal cells around the underlying dermis, the process known as epiboly, and the appearance of abnormal keratinization. In the condition of abnormal keratinization, or parakeratosis, the loss of cytoplasmic organelles may be incomplete, and residues of degenerate or pyknotic nuclei are present. In 1970 Sarkany and Gaylarde reported very early ultrastructural changes in adult human skin maintained in organ culture. Alteration of the normal keratinization process and migration of epidermal cells were observed during the first 48 hours. Ultrastructural studies of older cultures have been described by Prose *et al.* (1967), who examined epiboly and abnormal keratinization in cultures of adult human skin 6 days to 8 weeks old. Little is understood of the factors which lead to the structural alterations of skin *in vitro*, alterations which bear some resemblance to certain diseases of the epidermis *in vivo*, e.g. psoriasis. Basic knowledge in this direction would contribute towards a more useful experimental model and increase our understanding of normal growth and maturation.

In this report we describe growth conditions under which epiboly occurs. In synthetic medium containing whole or dialysed serum, epiboly is observed in explants of skin after 3 days' incubation. If, however, only the low mol. wt. components of serum are present in the growth medium, or no serum is added, epiboly is suppressed or completely absent up to the termination of a 19-day culture period. In the early stages of our work we attempted to suppress epiboly by treatment of the free edge of explants with solid CO_2. The criteria of epidermal growth and differentiation have been the healthy appearance and potential for

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DNA synthesis of the basal cells, the stratified organization of the epithelium and the development of a stratum corneum as judged by autoradiography and histological staining.

METHODS

Organ culture.—Specimens of adult human skin were obtained from amputated limbs or from patients undergoing plastic surgery. The donors were of either sex and included a wide range of ages. After swabbing the site (usually thigh) with aqueous ethanol and drying with cotton wool, the skin was removed with a Castroviejo keratotome set to cut at 0·5 mm thickness. Specimens obtained from plastic surgery patients were usually 0·25–0·3 mm thick. Excised sheets of skin were placed epidermal side down on sterile gauze soaked in Hanks' balanced salt solution (BSS) to which penicillin-G (200 units/ml) and streptomycin (100 μg/ml) had been added. Incubation of the explants was carried out within a few hours of removal of the skin.

A stainless steel leather punch was used to cut the skin into circular pieces. With this tool, the edges of the tissue were cut sharply and cleanly, with minimum trauma. The punch was equipped with 6 circular cutters varying in diam. from 2 mm to 5 mm. A larger cutter, 7·5 mm diam., was substituted in place of one of the smaller ones to increase the range.

Polythene sheeting, backed by thin cardboard, provided a base on which the skin was placed, dermal side down, during the cutting. The polythene sheeting, which was sterilized both by swabbing with aqueous ethanol and by u.v. light, served to prevent loss of moisture from the skin during the manipulations and aided in identification of the dermal side in the transfer of the explant. After incision with the punch, the specimen was removed from the cutter by inserting an orange stick into the opposite opening. The explant, separated from its backing, was then placed on a support prepared as described below.

A variety of supports was tested, including squares of cellulose pads and stainless steel wire or nylon mesh. Both wire and nylon mesh were more satisfactory than the cellulose pads, and nylon mesh supports were subsequently adopted for general use. These were made by gluing (with Araldite) a circle of nylon (400 mesh) to a piece of Teflon tubing (3 mm in length), internal diam. 1·6 cm. A second piece of tubing, 4 mm long, was cut into 8 sections; 3 of these were glued to the bottom side. Spaces between the feet were required to prevent trapping of air under the support. Millipore filter sheets (25 μ thick and 0·45 μ pore size) were cut slightly larger than the specimen and placed on the surface of the nylon mesh. The whole assembly was washed and sterilized in an autoclave. An assembly was inserted into a small, disposable Petri dish with sufficient growth medium to wet the filter sheets, usually 2·5 ml. A single circle of skin, either 2, 5 or 7·5 mm diam., was placed on top of the millipore filter and pressed gently down with a glass needle (made by closing the tip of a Pasteur pipette in a hot flame). Thus, close and even contact between the skin and filter was ensured. The growth medium reached the surface of the support, but did not cover the explant. Incubation was at 32°C in an atmosphere of 5% CO_2 in air. A tray of saturated NaCl solution at the bottom of the incubator provided a relative humidity (RH) of 75%.

Culture media.—The basic synthetic medium was Eagle's Minimal Essential Medium (MEM) (Wellcome Research, Beckenham, Kent) to which the antibiotics penicillin and streptomycin were routinely added. Further additions to the growth medium were tested, including calf serum, glutamine or human serum components. MEM with low mol. wt. components of human serum was prepared by dialysing fresh human serum directly into the synthetic medium under sterile conditions. Well-washed, boiled dialysis tubing was knotted at one end, while the other end was threaded through the hole in a white silicone rubber stopper. A length of narrow bore nylon or Teflon tubing was inserted through the stopper into the dialysis bag. The serum was introduced through the tubing by means of a syringe and long needle, after which the bag was lowered into a flask containing MEM. When 10 volumes of serum were dialysed against 90 volumes of MEM the medium was referred to as MEM plus 10% low mol. wt. components of serum. After exhaustive dialysis, 10 volumes of the dialysed serum from inside the bag were added to 90 volumes of fresh MEM, and the medium referred to as MEM plus 10% high mol. wt. components of serum.

Treatment of tissue before incubation.—It was at first thought that immobilization of the cut edges by freezing might suppress outward growth. For this treatment a cork borer, outer diam. equal to that of the specimen, was cooled in crushed CO_2 ice and applied to the outer edge of the tissue for a few seconds. In some cases the whole area of the tissue was frozen by means of a solid metal rod (diam. somewhat greater than the circle of skin) similarly cooled in CO_2 ice and applied to the tissue for a few seconds, after which the explant was incubated.

Histology and autoradiography.—Explants were removed at intervals and fixed in 10% neutral formalin. After dehydration the specimens were embedded in paraplast wax, and serial sections were stained with H. and E. Some sections were stained by PAS technique, before and after diastase treatment to demonstrate glycogen. Throughout the processing the millipore filter was left in place, as it did not interfere with the section cutting. During the culture period it served as a support, while, during the processing, it kept the tissue flat and provided a base for lifting the specimen without injury.

Some of the explants were labelled with tritiated thymidine (Thymidine-6-T, 13·7 Ci/mM/l. The Radiochemical Centre, Amersham) for 24 hours before harvesting. The activity of the growth medium was 1 μCi/ml. The only departure from the normal histological procedure up to the staining stage was the use of "subbed" slides, prepared by coating clean slides in a solution of gelatin and chrome alum.

The stripping film technique was employed, using Kodak AR 10 nuclear emulsion. The number of basal and suprabasal cells with labelled nuclei per specimen (4 μm thick, 4 mm length) was estimated in order to assess DNA synthesis.

OBSERVATIONS

The following observations were the result of examination of about 250 explants incubated for periods ranging from 1 to 19 days.

The condition of the skin was not affected by the size of the specimen within the range 2 mm to 7·5 mm, although the smallest pieces were difficult to manipulate and the largest tended to curl up and were wasteful of our limited supply. Consequently, for most of the work, explants were cut at 5 mm diam.

Response to Components of Human Serum

Incubation 3–5 days.—The general architecture of the epidermis as observed at the light microscope level was largely preserved during this period (Fig. 2). Epidermal cells often increased in size and there was usually some increase in number. Vacuoles were observed in many cells, and the nuclear material was generally more condensed than in controls of fresh skin (Fig. 1). A few nuclear bodies were visible in most cultured cells, whereas, in the control, the nuclear material was more granular and diffuse. There was, too, a general loosening of intercellular bonds in cultured skin. In the explants grown in MEM to which 10% human serum or the high mol. wt. components of the serum had been added, a short tongue of epithelial cells had migrated outward around the dermis. No such migration was observed in explants grown in MEM without serum or containing low mol. wt. components of serum. Autoradiographs of thymidine incorporation during the first few days of culture generally showed few labelled cells, regardless of the type of medium.

Incubation 6–8 days.—Although by this time some areas displayed a loss of organization, many regions appeared quite normal. There was usually some increase in the thickness of the epidermis, more pronounced in the cultures grown in MEM with 10% whole serum or high mol. wt. components. Advanced epiboly was observed in these specimens (Fig. 3b). No migration of cells could be seen in cultures maintained in MEM only or MEM supplemented with low mol. wt. components of serum (Fig. 3a). Some vacuolization and pyknotic nuclei were found in all cultures. The stratum corneum (SC) by now consisted of 2 layers, an outer basket weave layer and an inner layer of parakeratotic cells. The extent of abnormal keratinization was greater in cultures grown in 10% serum or high mol. wt. components of serum with many degenerate nuclei. In some specimens an increased incorporation of thymidine into basal and suprabasal cells occurred at this time, although usually this increase appeared when the cultures were some days older.

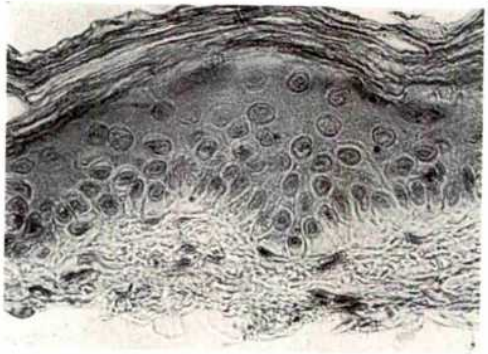


FIG. 1.—Section of normal adult human thigh skin. (H. and E. ×375.)

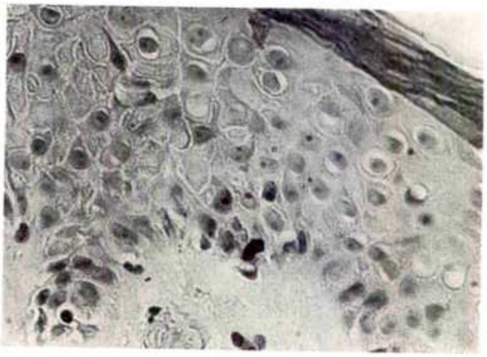
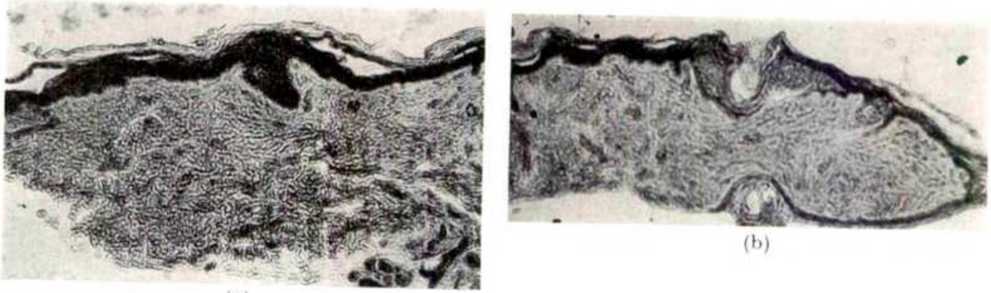


FIG. 2.—Explants harvested after 5 days of *in vitro* growth in MEM only. There is a general thickening of the epidermis and vacuolization, but the architecture is largely preserved. (H. and E. ×375.)



(a)

(b)

FIG. 3.—Explants harvested after 6 days of *in vitro* growth in (a) MEM plus low mol. wt. serum components, (b) MEM plus high mol. wt. serum components. In (a) there is no growth around the underside of the dermis, whereas, in (b), migration around the dermis (epiboly) is well advanced. At 10 days' incubation epiboly is usually complete in media of (b). (H. and E. ×45.)

Incubation 8–10 *days*.—By now there were generally fewer malpighian cells, and these were elongated and flattened parallel to the surface of the dermis, although surviving "pockets" of normal appearance remained. In some specimens grown in 10% serum the earlier increase in the number of cells had, by now, been compensated by the subsequent reduction so that the thickness of the malpighian layer was similar to that of the original epidermis. Preservation of the general structure was better in cultures grown in MEM containing low mol. wt. components of serum, little or no epiboly appearing in these cultures. Some newly keratinized areas presented a compressed, solid, eosinophilic structure in contrast to the open-weave appearance of the original SC.

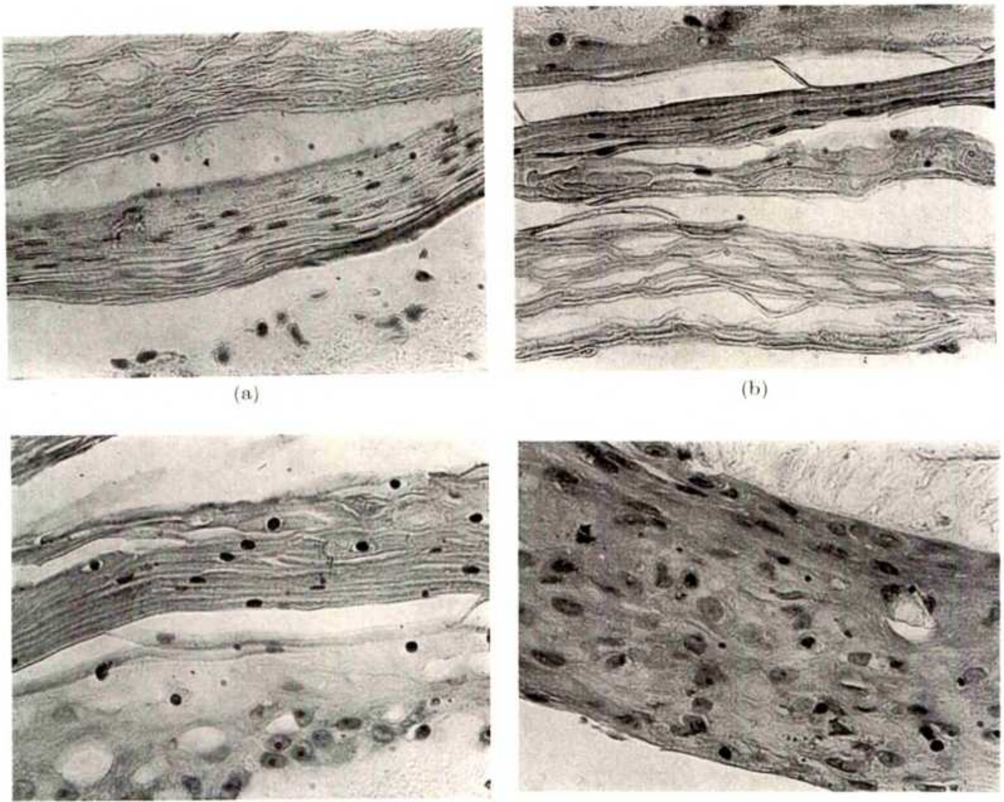


FIG. 4.—Explants after 14 days of incubation in (a) MEM only, (b) MEM plus 10% high mol. wt. serum components, (c), (d), MEM plus 10% whole serum. In (a) there are few malpighian cells remaining beneath the 2 separated keratinized layers. The parakeratotic layer in (b) is thick with numerous nuclear remnants, while in (c), in addition to the thick parakeratotic layer similar to (b), there are more surviving malpighian cells than in (a) or (b). In (d) the thick layer of epiboly is observed along the underside of the dermis. (H. and E. × 375.)

Cultures grown in 10% whole serum or high mol. wt. components of serum showed a greater degree of disorganization with a thick parakeratotic layer containing many pyknotic nuclei. Migration around the dermis was usually complete, the migratory layer thickened and parakeratotic.

Incubation 12–14 *days*.—A general disorganization was apparent in all cultures with 2 or 3 layers of elongated and flattened malpighian cells parallel to the dermal surface (Fig. 4a,b). The SC was still in 2 layers, the inner parakeratotic layer relatively thicker than the original outer basket-weave layer. Some differences were apparent, however. In the cultures grown in MEM with low mol. wt. serum components, the parakeratotic layer was more compressed and generally showed fewer nuclear remnants. In some specimens grown in MEM plus 10% whole serum some regeneration of epidermal cells was seen (Fig. 4c) and there were several layers of migratory cells along the millipore filter as well as a thick multicellular layer along the underside of the dermis (Fig. 4d).

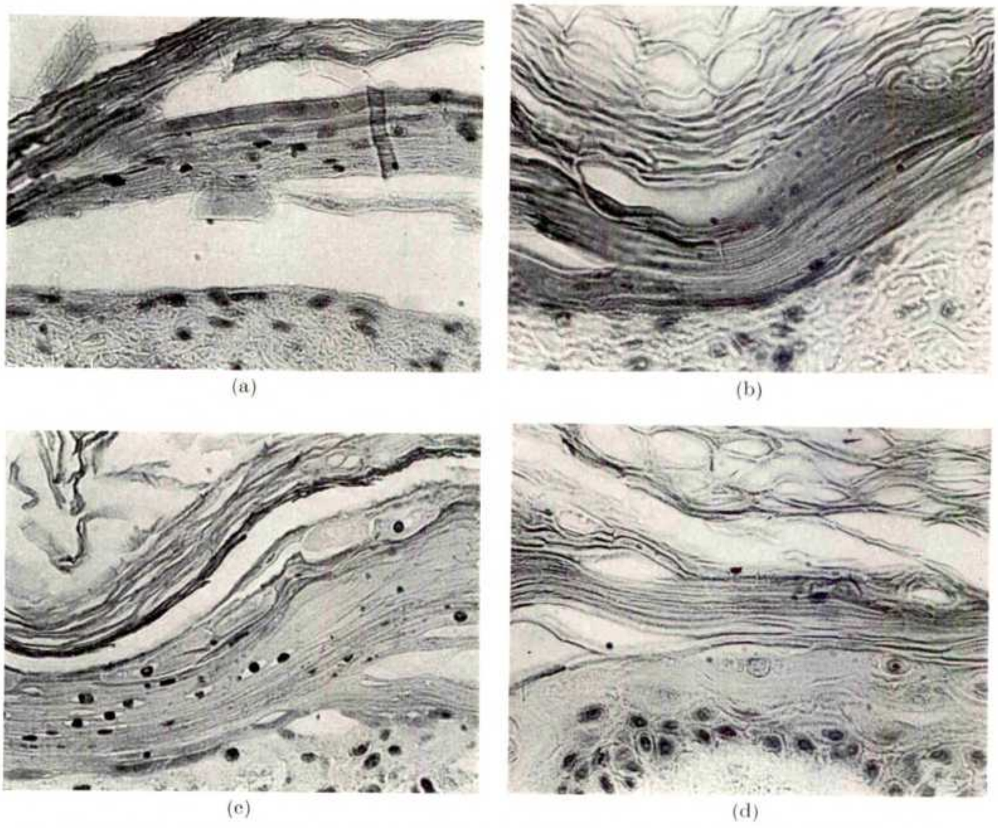

(a) (b) (c) (d)

FIG. 5.—Explants after 19 days of incubation in (a) MEM only, (b) MEM plus 27% low mol. wt. serum components, (c) MEM plus 10% high mol. wt. serum components, (d) MEM plus 10% whole serum. In (a) there are few malpighian cells remaining. In (b) a compressed parakeratotic layer is observed beneath the original SC. An extensive parakeratotic layer beneath the original SC is observed in (c). In (d) regenerated epithelial cells can be seen beneath the keratinized layer. (H. and E. ×375.)

There was no incorporation of thymidine in cultures maintained in MEM alone, whereas DNA synthesis was generally increased in the cultures grown in MEM to which serum components had been added.

Incubation 19 *days*.—Cultures grown in MEM only had degenerated to a parakeratotic layer separated from the dermis, with few malpighian cells to be found (Fig. 5a). The explants in MEM plus 10% high mol. wt. components had a thicker parakeratotic layer, but few malpighian cells (Fig. 5c). Cultures in MEM containing 27% low mol. wt. components also had a parakeratotic layer, which was more compressed, with fewer nuclei present (Fig. 5b). In addition a few epithelial cells appeared to be growing between the 2 layers of SC. Below the parakeratotic layer was a thin layer of flattened malpighian cells.

The most striking changes were observed in cultures grown in MEM plus 10% whole serum (Fig. 5d). The epidermis had regenerated, showing well-organized columnar basal cells and a keratinized layer with few residual nuclei. Epiboly was complete and, in some regions, the explant was divided into small islands of dermis by invading epidermis. The migrated cells had also given rise to a healthy-appearing epidermis.

Incorporation of thymidine was absent in cultures grown in MEM alone, while an increased uptake of thymidine was observed in explants grown in MEM containing either serum components or 10% whole serum. The increased DNA synthesis found in cultures grown in MEM plus 10% whole serum corresponded with the time of regeneration of epidermis.

TABLE I.—*Number of Basal and Suprabasal Cells with Labelled Nuclei per Specimen (4 μ thick, 4 mm length). Average of 5 Specimens*

	Incubation time (days)								
Growth medium	1	2	3	5	8	10	12	14	19
MEM only	—	—	—	2	6	—	0	0	0
MEM + 11% *low mol. wt. serum	—	—	—	0	—	2	0	—	—
MEM + 20% low mol. wt. serum	—	—	—	1	0	—	20	—	—
MEM + 27% low mol. wt. serum	—	—	—	17	22	—	84	—	—
MEM + 10% high mol. wt. serum	—	—	—	1	6	3	—	13	28
MEM + 10% whole serum	10	1	0	2	2	9	16	0	23

* See text on preparation of medium containing low molecular weight (mol. wt.) components of serum.

Table I shows the incorporation of thymidine into basal and suprabasal cells, measured over the 19-day culture period for the different media. Incorporation appeared to increase in long term incubations in media containing serum or serum components, but not in medium of MEM alone.

Treatment With Solid CO_2

The effect of treatment by freezing the outer surface of the specimen with solid CO_2 was striking. At 3 days the organization of cells at the treated edge was completely preserved. The epidermis, however, had separated from the dermis over the treated area. Much of the intracellular material had disappeared, and staining was very poor. There was no migratory movement of epidermal cells from the edge. At 5 days, however, in some explants, migration had started from the untreated tissue adjacent to the edge so that freezing of the edges of the tissue, while delaying epiboly, may not prevent it. Central areas of the tissues were similar in appearance to untreated, control specimens.

When the whole area of the explant was frozen for a few seconds and then incubated, complete immobilization of the tissue occurred, with no signs of any growth or maturation. There was no migration of cells and the structure of the epidermis remained well preserved and static up to the termination of a 7-day culture period (Fig. 6b). Nuclei were nearly empty and poorly stained.

The absence of growth in the frozen specimens was confirmed by autoradiography. No labelling could be detected after 24 hours pulse with tritiated thymidine, either in the early or later cultures incubated for 7 days, whereas at 7 days the untreated specimen exhibited the usual loss of organization, the flattened malpighian cells and abnormal keratinization, as already described, and an accompanying increased DNA synthesis in the basal and suprabasal layers (Fig. 6a).

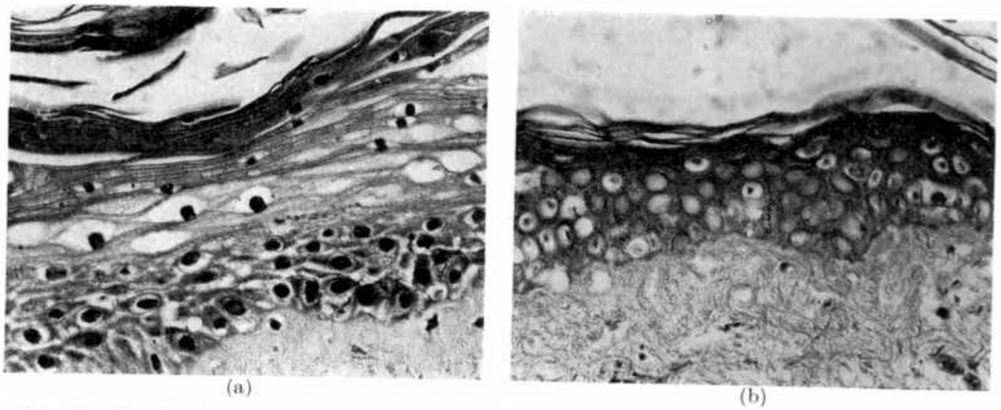

(a) (b)

FIG. 6.—Explant harvested after 7 days *in vitro* growth in MEM plus 10% whole serum: (a) untreated (b) pretreated by freezing with solid CO_2 over the surface before incubation. In (a) the expected changes have occurred, but in (b) there is no parakeratotic layer or flattening of the malpighian layer. Although nuclear constituents have been lost, the general structure appears unaltered. (H. and E. ×375.)

Presence of Glycogen

Controls of fresh, normal skin were glycogen-negative, with the occasional trace. At 5 days' incubation all explants showed glycogen-positive material in the cytoplasm of the malpighian layer. Thereafter the amount of glycogen decreased, its gradual disappearance corresponding with the flattening and thinning of the malpighian layer. Although leading cells of epiboly were glycogen-positive, the regenerated epidermal cells at 14 and 19 days' growth in MEM plus 10% whole serum were found to be glycogen-negative.

DISCUSSION

We find that epiboly does not occur in explants incubated in the absence of high mol. wt. components of serum. An interpretation of this finding is suggested by the observation that collagenases are produced by skin *in vitro* (Evanson, 1970). The resulting breakdown of dermal tissue could prevent the adhesion necessary for the migration of epidermal cells on the dermal surface. A potent inhibitor of collagenases is known to be present in normal serum. The inhibitor is a high mol. wt. protein, probably an α_2-macroglobulin or a protein associated with it.

The presence of serum or the high mol. wt. components of serum in the medium, by inhibiting collagenases, could promote the adhesion required by the migratory cells. The appearance of the dermis (Fig. 3a), where the explant was incubated in MEM in the absence of high mol. wt. components of serum, suggests a degree of breakdown greater than that observed in Fig. 3b, where incubation was in MEM containing high mol. wt. components of serum.

In all cultures incubated for periods beyond 5 days there was a parakeratotic layer beneath the original SC. The presence of high mol. wt. components of serum in the medium enhanced both the degree of abnormal keratinization and the thickness of the layer, which generally showed more pyknotic nuclei than observed in specimens cultivated in MEM containing low mol. wt. components of serum. The appearance of an eosinophilic, solid-stained structure beneath the original basket weave SC is discussed by Spearman (1970) in connection with the parakeratotic cells of human skin lesions and is attributed to the lesser degree of breakdown of structural components in the keratinized cells. In the normal SC the breakdown and subsequent leaching out of intracellular components leads to the appearance of spaces, whereas in parakeratosis the denser structure is retained.

Incorporation of thymidine was generally observed in long-term cultures grown in media containing serum or components of serum. In MEM alone, however, no incorporation was detected after about 12 days' incubation. The use of unsupplemented medium is desirable in experiments to investigate the effects of various additives. Where a completely defined medium is indicated, cultures maintained in MEM would be useful.

Regeneration of the epidermis occurred in the presence of 10% whole serum. The factors involved are undoubtedly complex, and an adequate explanation at this time is not possible. One requirement is the adhesion of the basal cells to the dermis. The basal cells must be closely connected with dermal papillae, although a limited growth can occur along the millipore filter. There is evidence for an induction factor synthesized by dermal fibroblasts, a factor which is a prerequisite for regeneration of epidermis (Dodson, 1967). It may be possible to promote regeneration in organ cultures by the renewal of dermal factors, perhaps in the form of dermal powder or extract (Briggaman, 1971).

Epidermal growth may also be stimulated by the removal of the keratinized layer, which becomes loosened in long-term incubations. A main factor in the epidermal control mechanism is thought to be the epidermal chalone, a protein isolated from the epidermis and shown to act as a tissue-specific mitotic inhibitor (Bullough, 1970). The loosening of the top layers, by removal of the inhibitor, may stimulate mitotic activity. It has been shown (Reaven, 1968) that stripping of the SC before incubation stimulates mitotic activity, the increased activity occurring 3–4 days after the stripping period. Attempts by the same workers to strip the SC during culture were unsuccessful.

The effect of freezing an area of epidermis resulted in a localized cessation of growth and maturation, the structure remaining static, with gradual loss of nuclear constituents. The application of such treatment may be of value in studies where it is desirable to halt metabolic processes, for example in the study of membrane interaction in transport phenomena or in permeability studies of metabolizable substrates.

It was of interest to find that the malpighian cells of cultured explants were glycogen-positive, the glycogen disappearing after differentiation to a parakeratotic layer had occurred. Glycogen is known to reappear in pathological conditions and is a feature of certain tumours. The leading cells of epiboly were also glycogen-positive, but the regenerated epidermal cells, after 19 days' incubation in MEM containing 10% human serum, showed no glycogen, behaving in this respect as the mature normal epidermis in which little or no glycogen is present.

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INCREASED *IN VITRO* RADIO-SENSITIVITY OF MALIGNANT MELANOMA INDUCED BY THE *IN VIVO* ADMINISTRATION OF CHLORPROMAZINE

MICHAEL COOPER AND YUTAKA MISHIMA

Summary.—*In vitro* x-ray irradiation with 3000 R of strips of Fortner's melanotic melanoma was found to have no effect on the rate of successful transplantation of these strips in hamsters. However, when the hamsters bearing the melanomata from which the strips were obtained had been treated with 3·5 mg chlorpromazine before irradiation, the incidence of melanoma transplantability was reduced to 33%. Chlorpromazine-treated melanomata which did grow after irradiation grew at a greatly reduced rate. It is postulated that chlorpromazine may increase the radio-sensitivity of melanized cells by masking the free radical nature of melanin.

In vitro x-ray irradiation of pigmented and relatively non-pigmented tissue slices of Cloudman S91 melanoma has indicated a relative radio-resistance of the pigmented tissue (Cobb, 1956). Further studies, using the inactivation of enzymes with u.v. irradiation as the test system, have also indicated a protective effect of melanin (Seiji and Itakura, 1966). This radio-protective effect has been interpreted to be a major factor in the radio-resistance of melanotic malignant melanoma (Ackerman and Regato, 1962). It has been postulated (Greenberg and Kopac, 1963; Mason *et al.*, 1960; Seiji and Itakura, 1966) that the radio-protective effect of melanin is due to its stable free radical nature (Mason *et al.*, 1960), enabling it to capture radiation-induced free radicals and thus spare critical cell molecules. If this is the case, it should be possible to dampen this protective effect of melanin by saturating its free radicals.

Chlorpromazine is able to bind with a high degree of selectivity to melanin (Blois, 1965), and available evidence indicates that this binding is due to the formation of a charge transfer complex involving free radicals formed by chlorpromazine, and melanin (Potts, 1964). In addition, the electron spin resonance signal of melanoprotein is reduced upon addition of chlorpromazine (Bolt and Forest, 1966). Therefore, the effect of chlorpromazine on the radioresponse of malignant melanoma was investigated.

MATERIALS AND METHODS

Fortner's melanotic melanoma No. 1 (Fortner *et al.*, 1961), obtained from J. G. Fortner in 1964 and carried in our laboratory by subcutaneous implantation in Syrian (golden) hamsters, was used in this study. Chlorpromazine HCl (CPZ), generously supplied by

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Shionogi Pharmaceutical Co., was daily injected subcutaneously into 2 hamsters on the opposite side to that of the implantation, beginning the day following tumour transplantation. The initial dose was 0·25 mg per hamster (60–80 g), and was gradually increased to 1·0 mg over a 7-day period. At the time of tumour excision, 8 days after implantation (1 day after the CPZ treatment) a total of 3·5 mg CPZ had been administered. X-ray irradiation was performed following the method of Cobb (1956). A portion of the actively growing periphery of the tumour was removed, cut into small strips approximately 1 × 1 × 2 mm, and suspended in 1·0 ml saline containing one drop procaine penicillin-G (200,000 units/ml) and streptomycin sulphate (0·25 g/ml solution. The tissue strips were irradiated in a standard glass liquid scintillation vial without top, in ice, by a Dermopan type x-ray unit (factors: 20 cm distance, 43 kV, 0·6 mm Al filter, irradiation time 12·8 minutes), with 3000 R. One strip each was then implanted subcutaneously by trocar to each of 15 hamsters. As controls, 40 hamsters received non-irradiated similar implants from 6 non-CPZ-treated donors; 10 hamsters received similarly irradiated implants except that no CPZ was given to the donor animals; 5 hamsters received non-irradiated implants derived from CPZ-treated hamsters. The interval between removal of the tumour and the last implantation was less than 1 hour, and tumour strips from CPZ-donors were always implanted before control tumour strips. All animals were observed for 2 months to determine the success of transplantation (Cooper and Mishima, 1971).

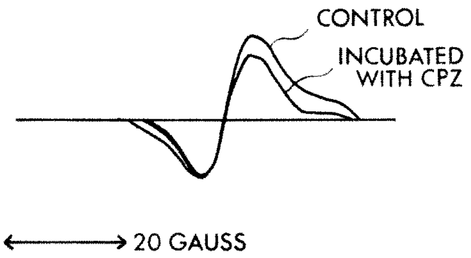
RESULTS AND DISCUSSION

The effects of CPZ and irradiation on melanoma transplantability are shown in Table I. Of 40 control hamsters receiving non-irradiated similar implants from 6 non-CP2 treated animals, and 5 control hamsters receiving non-irradiated implants derived from CPZ-treated donors, all developed tumours within 2 weeks. Of 15 hamsters receiving irradiated melanoma implants which had been donated from hamsters receiving CPZ, only 5 developed melanomata. Of 10 control hamsters receiving irradiated melanoma implants from donors which did not receive CPZ, all 10 developed melanomata (statistical significance based on student's *t* test: $P < 0{\cdot}01$). Those 5 CPZ implants which did grow, grew at a significantly slower rate, averaging 3·3 cm in greatest diameter after 34 days compared to 5·0 cm in the irradiated controls ($P = 0{\cdot}01$). No difference in transplantability between the non-irradiated CPZ-treated and non-treated implants was observed in this experimental system. Since the completion of these experiments, the findings of Barth *et al.* (1968), showing increased radiosensitivity of mouse Crocker-sarcoma 180 after treatment with chlorpromazine, have come to our attention. It has also been shown that chlorpromazine is

TABLE I.—*Transplantability of* in vitro *Irradiated and Non-irradiated Tissue Strips of Fortner's Melanotic Melanoma with and without Chlorpromazine Pretreatment, Showing Increased Radio-sensitivity of Melanoma Implants Derived from CPZ-treated Animals*

	Number of viable transplants		
	CPZ to donor	Without CPZ to donor	*P*
Without irradiation	5/5	40/40	
3000 R before transplantation	5/15	10/10	< 0·01

able not only to increase radio-sensitivity of this sarcoma, but also to inhibit growth of various unirradiated tumours, either alone or in combination with other drugs (Belkin and Hardy, 1957; Gottlieb *et al.*, 1960; Maguire, 1968; Van Woert and Palmer, 1969), despite its ineffectiveness for many others (Chorzy, 1959; Cranston, 1958). While the exact mode of action of CPZ on these various tumours remains uncertain, the generally accepted view that the stable free radicals of melanin function in a radio-protective capacity (Greenberg and Kopac, 1963; Mason *et al.*, 1960; Seiji and Itakura, 1966) and the finding of a reduced paramagnetic signal of purified melanin after treatment with CPZ (Fig. 1), seems to indicate that the reported radio-sensitizing effect of chlorpromazine on melanotic malignant melanoma is due, at least partially, to its ability to mask the stable free radicals of melanin.



E.S.R. signal of purified melanoma melanin after incubation with chlorpromazine. Factors: 3365 ± 100 G; att. −12·5 dB; gain 1 × 200; mod. 1 G; chart 30 mm/min.

FIG. 1.—The electron spin resonance signal of Fortner's melanotic melanoma melanin granules purified by hydrolysis using 6 N HCl at 100°C for 24 hours is reduced approximately 25% after the addition of CPZ (1 mg/ml) with exposure to the light from a bromine vapour lamp for 5 min. The CPZ was removed by washing prior to electron spin resonance spectroscopy.

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CORRELATION BETWEEN VARIOUS PROTEINS OF BOVINE SNOUT EPIDERMIS*

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Summary.—A comparison is made of several properties of urea-extractable proteins which were isolated from mammalian epidermis. A 6 mol/l urea solution extracts from epidermis at least 3 proteins which are precipitated maximally at pH 6·3, 5·5 and 4·5. The urea 4·5 and 5·5 proteins are soluble in water at pH 7·5 to 8·5. The urea 6·3 proteins were rather insoluble in the absence of urea, but were partially solubilized by 0·05 N NaOH to yield protein which precipitated maximally at pH 5·5, the alkali-soluble 5·5 protein. Also, the —S—S— bonds of the urea 6·3 proteins were reduced with Clelands reagent (dithiothreitol) and the resultant free-SH groups were reacted with iodoacetate to yield soluble- and insoluble-*S*-carboxymethyl derivatives of these proteins. The urea 6·3 and alkali-soluble 5·5 proteins have molecular weights exceeding 20×10^6, whereas the *S*-carboxymethyl proteins have a molecular weight of $2{\cdot}2 \times 10^5$. The urea 5·5 protein extracted from epidermis at room temperature, and a portion of the urea 5·5 protein extracted at 0°C, have a molecular weight of $5{\cdot}0 \times 10^4$. With antisera prepared in rabbits against the urea 5·5 and 6·3 proteins, the following proteins were found to have common antigenic determinants: urea 4·5, 5·5 and 6·3 proteins, alkali-soluble 5·5 protein, and the *S*-carboxymethyl-soluble proteins.

SINCE the original investigations of Rudall (1952) on the urea-extractable proteins of mammalian epidermis, many investigators have isolated various proteins from this tissue by different extraction procedures (Bernstein *et al.*, 1970; Baden *et al.*, 1968; Carruthers, 1970; Carruthers *et al.*, 1955, 1957; Crounse, 1963; Matoltsy, 1965; Rothberg *et al.*, 1965). Concentrated urea solutions extract from epidermis proteins which precipitate maximally at pH 4·5, 5·5 and 6·3 (Rudall, 1952; Bernstein *et al.*, 1970; Carruthers *et al.*, 1955). The relationships between these urea-extractable proteins are not known at the present time. In this report an attempt is made to obtain some information on this subject.

MATERIAL AND METHODS

Beef snouts were obtained at a slaughter house and kept in an ice bath until they were returned to the laboratory. The thick epidermis was sliced from the snouts with a Brown Electro-Dermatome. The epidermis was placed in a 6 mol/l urea solution (50 ml/g) and shredded with a Waring Blender. The epidermis was then extracted at 0–4°C or at 25°C for 1 week by continuous stirring (magnetic stirrer) according to the procedure of Rudall (1952). Details of the modification in this procedure have been

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given (Carruthers *et al.*, 1955, 1957; Carruthers, 1970) and are outlined in Fig 1. Briefly, the 6 mol/l urea extract was centrifuged at 600 × g for 15 minutes to remove residue of epidermis not extracted by urea The extract was then filtered through a coarse sintered-glass filter to remove any remaining tissue residue.

The filtrate was dialysed against water at pH 7·0–7·4 to remove the urea and then centrifuged at 20,000 × g for 20 minutes to remove any insoluble proteins. The pH of the dialysed solution was then lowered carefully by the addition of 1 N HCl at 25°C. At pH 6·3, maximum precipitation of sticky and fibrous proteins resulted. These proteins (called the urea 6·3 proteins) were removed by centrifugation at 500 × g for 15 minutes at 25°C. The pH of the supernatant fraction obtained from the 6·3 protein precipitation was lowered to 5·5, at which pH the urea 5·5 protein precipitated maximally. The urea 5·5 protein was removed by centrifugation at 500 × g for 15 minutes and this protein is called the urea 5·5 protein-0 (i.e. extracted at 0°C). The pH of the supernatant fraction was then lowered to 4·5, at which value the urea 4·5 protein

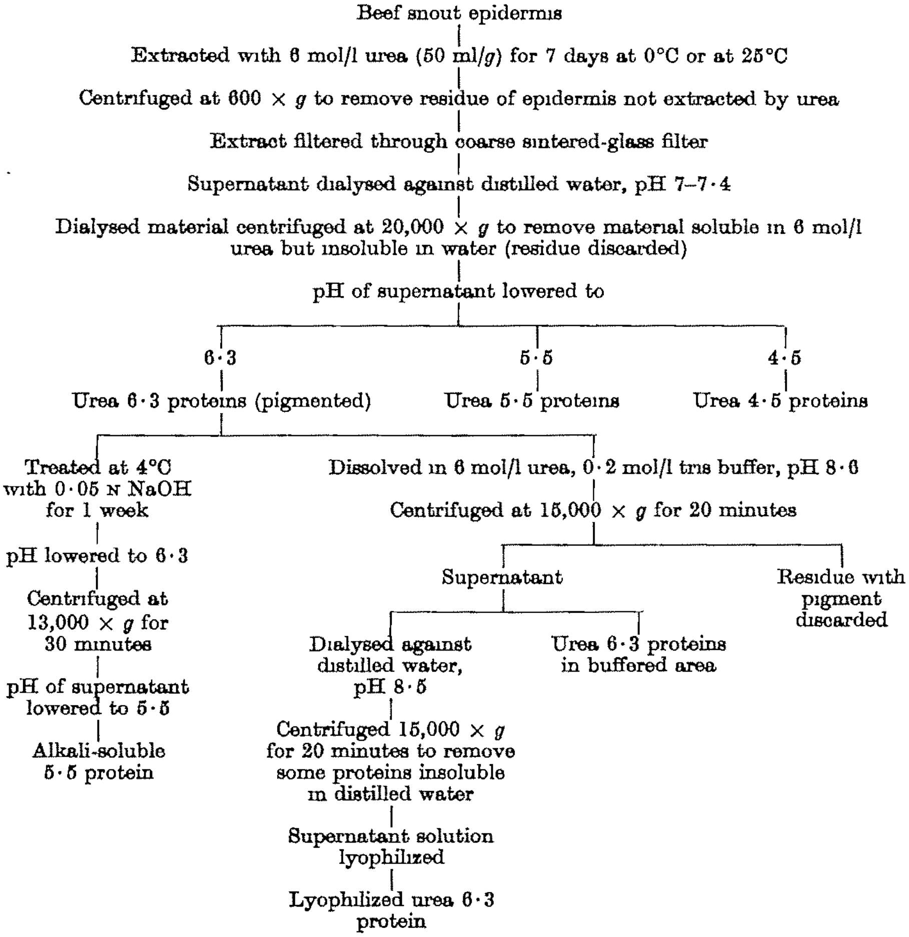
Beef snout epidermis
Extracted with 6 mol/l urea (50 ml/g) for 7 days at 0°C or at 25°C
Centrifuged at 600 × g to remove residue of epidermis not extracted by urea
Extract filtered through coarse sintered-glass filter
Supernatant dialysed against distilled water, pH 7–7·4
Dialysed material centrifuged at 20,000 × g to remove material soluble in 6 mol/l urea but insoluble in water (residue discarded)
pH of supernatant lowered to
6·3
5·5
4·5
Urea 6·3 proteins (pigmented)
Urea 5·5 proteins
Urea 4·5 proteins
Treated at 4°C with 0·05 N NaOH for 1 week
pH lowered to 6·3
Centrifuged at 13,000 × g for 30 minutes
pH of supernatant lowered to 5·5
Alkali-soluble 5·5 protein
Dissolved in 6 mol/l urea, 0·2 mol/l tris buffer, pH 8·6
Centrifuged at 15,000 × g for 20 minutes
Supernatant
Residue with pigment discarded
Dialysed against distilled water, pH 8·5
Urea 6·3 proteins in buffered area
Centrifuged 15,000 × g for 20 minutes to remove some proteins insoluble in distilled water
Supernatant solution lyophilized
Lyophilized urea 6·3 protein

FIG. 1.—Procedure for the extraction of proteins from epidermis with 6 mol/l urea.

precipitated maximally. The urea 4·5 protein was separated by centrifugation as above.

The urea 5·5 and 4·5 proteins were purified by the procedure of Rudall (1952) by dissolving them in alkaline water (pH 7·5 to 8·5), and by reprecipitation of the proteins at their isoelectric points. This procedure was carried out 3 times. The urea 4·5 and 5·5 proteins were finally dissolved in 0·05 mol/l barbital buffer, pH 8·6, and the protein content was determined by the procedure of Lowry *et al.* (1951). The urea proteins obtained by the extraction of epidermis with 6 mol/l urea at 25°C were also isolated and purified. These proteins are called the urea 4·5 and 5·5 proteins-25 (i.e. extracted at 25°C).

The urea 6·3 proteins (from the urea extraction at 0–4°C) were solubilized in 2 different ways (Fig. 1). These proteins were solubilized by stirring (magnetic) with 0·05 N NaOH at 0–4°C for 1 week (Woernley *et al.*, 1957). The pH of the solution resulting from this treatment was lowered to 6·3 at 25°C and the solution was clarified by centrifugation at 13,000 × *g* for 30 minutes to remove proteins which were not solubilized by NaOH. The pH of the supernatant fraction was then lowered by the addition of HCl, and maximum precipitation of a protein occurred at pH 5·5. This protein is called the alkali-soluble 5·5 protein. A small amount of protein also precipitated at pH 4·5 in the supernatant obtained from the 5·5 protein precipitation. These 2 proteins were purified in the same manner as the urea 4·5 and 5·5 proteins. The yields of the alkali-soluble 5·5 and 4·5 proteins were, respectively, 5% and 0·5% (wet weight of the 6·3 urea proteins). The yield of the urea 5·5 protein was 0·5% of the snout epidermis extracted (wet weight basis).

The urea 6·3 proteins were also solubilized by homogenization with 6 mol/l urea buffered at pH 8·6 with 0·2 mol/l tris or with 0·05 mol/l barbital. Following centrifugation at 15,000 × *g* for 20 minutes to remove pigment, the clear supernatant fraction containing the urea 6·3 proteins was used in this form. Also, the 6·3 protein-urea mixture was thoroughly dialysed against distilled water maintained at pH 8·6 to 9·0 and then centrifuged at 15,000 × *g* for 20 minutes to remove any water-insoluble proteins. This protein solution was used as such, following protein determination (Lowry *et al.*, 1951), or the solution was lyophilized (called lyophilized 6·3 protein). The urea 6·3 proteins were also dialysed against 0·05 mol/l barbital buffer, pH 8·6, with or without 0·1 mol/l NaCl for some purposes.

The available —S—S— bonds of urea 6·3 proteins were also reduced with Cleland's reagent* (dithiothreitol) and the free-SH groups produced were reacted with iodoacetic acid to form *S*-carboxymethyl derivatives of these proteins (Cleland, 1964). For this purpose the urea 6·3 proteins (60–120 mg) were solubilized in 8 mol/l urea buffered at pH 8·6 with 0·1 mol/l tris-acetate. The solution was placed in a 50 ml Erlenmeyer flask (glass-stoppered), and air was removed by passing N_2 gently over the surface of the protein solution for 15 minutes. Cleland's reagent was added to a concentration of 0·05 mol/l, and the solution was incubated at 38°C overnight. A drop of phenol red was then added to ascertain whether the protein solution was alkaline; if not, dilute NaOH was added to alkalinize the solution. Next, iodoacetate was added (to block the free-SH groups) to a concentration of 0·12 mol/l. The protein-iodoacetate solution was kept in the dark for 1 hour, after which the hydrogen-ion concentration was lowered to between pH 4 and 5. The *S*-carboxymethyl proteins were dialysed against several changes of 0·05 mol/l $NaHCO_3$ for 18 hours. Small amounts of insoluble proteins were removed from the *S*-carboxymethyl proteins by centrifugation at 6000 × *g* for 15 minutes. About 60% of these *S*-carboxymethyl proteins were less soluble than the original urea 6·3 proteins, whereas some 40% became more soluble than the urea 6·3 proteins. The insoluble *S*-carboxymethyl proteins were soluble in pH 8·6 buffered 6 mol/l urea or in 6 mol/l urea alone.

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* Personal communication from Dr B. K. Seon of this Institute on the procedure employed for reduction of the urea 6·3 proteins.

Preparation of antisera

Antisera were prepared in rabbits against the urea 5·5-0 and 6·3 proteins; the latter were extracted at 0–4 °C. These protein solutions in 0·05 mol/l barbital buffer, pH 8·6, were emulsified with Freund's complete adjuvent by homogenization in an all-glass homogenizer. Each ml of emulsion contained 2 mg of 5·5 or 6·3 urea proteins. For each protein, 3 ml of emulsion were injected intradermally into some 30 sites on the backs and sides of each of 3 rabbits, and also several injections were made into the foot pads. The rabbits were bled from the ear veins in 2 weeks, and weekly thereafter as long as the titres were good, as determined by the development of strong precipitin bands by immunodiffusion in agar with a fixed amount of antisera and graded levels of antigens All 6 rabbits responded well to the proteins so that antisera were withdrawn by ear bleeding for a period of 10 weeks. The antiserum from each rabbit was pooled and maintained at −30 °C.

The antisera were used unabsorbed and adsorbed with either bovine serum albumin or with the latter and then with beef kidney and liver sediments to ensure complete removal of antibodies which might have developed against non-epidermal tissue components. For this purpose, 3 ml of each antiserum were adsorbed with 0·15 ml of bovine serum and the antisera were allowed to stand overnight at 4 °C. Any precipitate was removed by centrifugation at 15,000 × *g* for 10 minutes. The bovine serum-treated antisera were then adsorbed twice with bovine liver sediments and once with kidney sediments. (Washed sediments for each adsorption were prepared from 10 ml of 20% physiological saline homogenates of beef liver and kidney.) Sediments were separated from the antisera by centrifugation at 15,000 × *g* for 10 minutes.

Immunodiffusion

Immunodiffusion experiments were carried out on microscope slides in 1·5% agar buffered with barbital, pH 7·6, and containing 7·5% glycine to help maintain the solubility of the epidermal antigens, and 0·1% NaN_3 as preservative. The centre well contained 30 μl of the antisera, either pretreated with 5 μl of bovine serum, or adsorbed with the latter and with liver and kidney sediments. The peripheral wells contained 120 μg of the various epidermal antigens. After application of the antisera and antigens, the slides were developed in a moist atmosphere for 1 week. The slides were then washed twice with saline and for 1 hour with distilled water, covered with paper strips, and dried. Buffalo black was used to stain the bands of precipitation.

Chromatography

Chromatography of the various epidermal proteins was carried out principally with Sephadex G200 and Sepharose 4B, with conventional columns as indicated in the figure captions. Upward flow chromatography was employed, when epidermal proteins were dissolved in concentrated urea solutions. For this purpose a Pharmacia laboratory column K 25/45 in conjunction with an LV–3 valve and Buchler micropump were used. Three ml fractions were collected from the columns and the protein content was determined by measuring the absorption at 280 nm or by the procedure of Lowry *et al.* (1951). Blue Dextran 2000 was used to measure the column void volume, V_0. Molecular weight determinations were derived from a calibration curve prepared with pure proteins (ribonuclease A, chymotrypsinogen, ovalbumin, desoxyribonuclease, bovine serum albumin, and γ-globulin) on Sepharose 4B by the method of Marrink *et al.* (1969), and on the same material by upward flow chromatography in the presence of 6 mol/l urea Protein samples were dissolved either in 0·05 mol/l barbital buffer, pH 8·6 containing 0·1 mol/l NaCl, or in this solution containing 6 mol/l urea for conventional and upward flow chromatography respectively. The appropriate solution was used for eluting the proteins from the columns.

Amino-acid analyses were carried out on samples of proteins which were hydrolysed in 6 N HCl under nitrogen for 20 hours at 108°C in sealed ampoules. Quantitative amino-acid analyses were performed on a Technicon automatic amino-acid analyser, using a 6·5 h accelerated gradient with norleucine as an internal standard (Roholt *et al.*, 1967). Under these conditions of hydrolysis tryptophan is destroyed, and there is a 5% destruction of threonine and 10% destruction of serine.

RESULTS AND DISCUSSION

Immunodiffusion

The immunodiffusion patterns are shown in Fig. 2. In (a), antisera raised against the urea 6·3 proteins reacted with the urea 6·3 (**A**), alkali-soluble 5·5 (**B**), and the urea 5·5-0 (**C**) proteins to yield 2 precipitation bands of identity with the 3 proteins. Also, the urea 4·5-0 and 4·5, and 5·5 proteins-25 and **D**, the *S*-carboxymethyl-soluble derivatives of the urea 6·3 proteins, gave precipitin bands of identity with these antisera and antigens **A**, **B** and **C** (not shown). The *S*-carboxymethyl-insoluble proteins were completely soluble only in buffered or unbuffered 6 mol/l urea solutions and were not tested by immunodiffusion. In (b) antisera prepared against the urea 5·5 proteins-0 also gave precipitin bands of identity with the urea 5·5-0 (**C**), urea 6·3 (**A**), alkali-soluble 5·5 proteins (**B**), and with the *S*-carboxymethyl-soluble derivatives of the urea 6·3 proteins (**D**). Similarly, the urea 4·5-0 and 4·5 and 5·5 proteins-25 gave precipitin lines of identity with these antigens and the same antisera (not shown). These immunodiffusion patterns suggest that the high molecular weight 6·3 urea proteins, the alkali-soluble 5·5 protein obtained from the urea 6·3 proteins, and the low molecular weight urea 4·5 and 5·5 urea proteins-0 and -25 have common antigenic determinants.

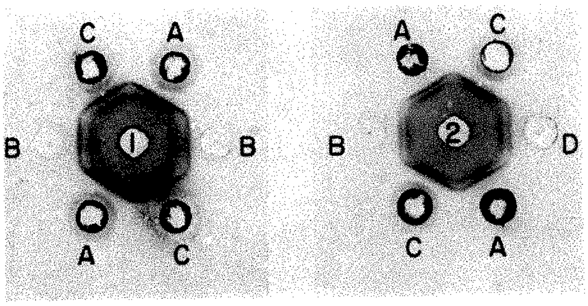

FIG. 2.—Agar gel precipitation patterns of antisera prepared against (a), the urea 6·3 proteins, and against (b), the urea 5·5 proteins-0 of epidermis. The following abbreviations are used for the various epidermal antigens: **A**, urea 6·3 proteins; **B**, alkali-soluble 5·5 protein; **C**, urea 5·5 proteins-0; **D**, *S*-carboxymethyl-soluble derivatives of the urea 6·3 proteins.

Chromatography

The alkali-soluble 5·5 protein was excluded from columns of Sephadex G200 and Sepharose 4B, thus indicating that this protein has a molecular weight exceeding 20×10^6, the exclusion limit of Sepharose 4B. The alkali-soluble 5·5 protein dissolved in 6 mol/l urea buffered at pH 8·6 with 0·05 mol/l barbital buffer containing 0·1 mol/l NaCl was also excluded by Sepharose 4B in upward flow chromatography with no evidence of proteins which were retarded. The

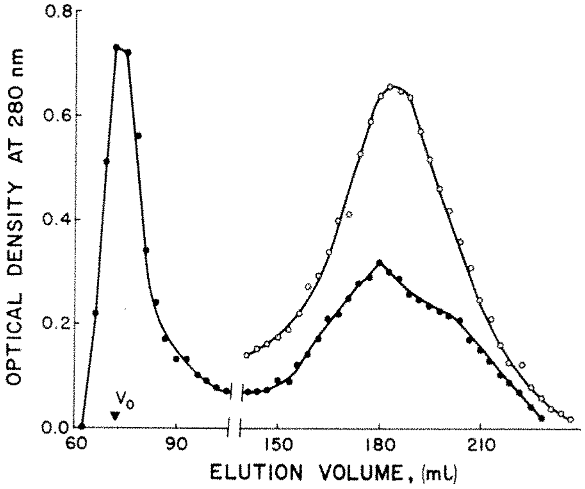

Fig. 3.—Elution profiles on Sephosear 4B (bed dimensions 2·8 cm × 39 cm). V_0, the void volume, was 72 ml and the flow rate was 18 ml/hour. The protein solutions were dialysed overnight against 0·05 mol/l barbital buffer, pH 8·6, containing 0·1 mol/l NaCl in Fig. 3 and 4; the same solution was used to elute the proteins. ●——● Urea 5·5 proteins-0; ○——○ Urea 5·5 proteins-25.

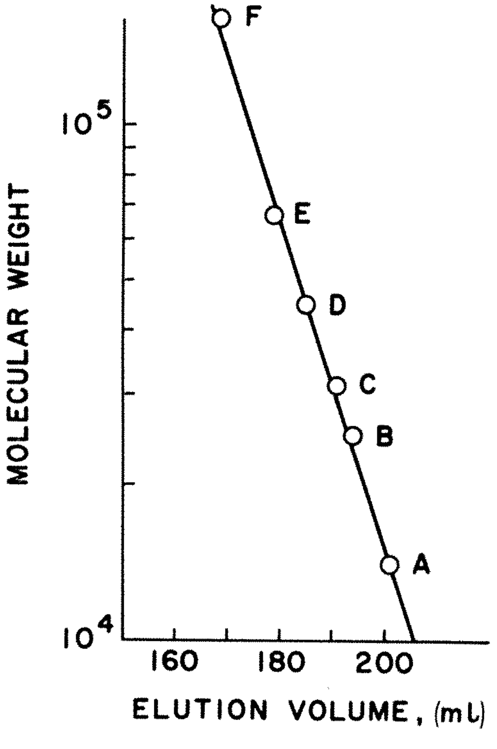

Fig. 4.—Molecular weight as a function of the elution volume. Molecular weights of **A**, ribonuclease A; **B**, chymotrypsinogen; **C**, desoxyribonuclease; **D**, ovalbumin; **E**, bovine serum albumin; and **F**, γ-globulin were plotted against the elution volume after chromatography on Sepharose 4B (bed dimensions, flow rate and V_0 were the same as in Fig. 3).

alkali-soluble 5·5 proteins may have been altered considerably by the alkali procedure used for its extraction. This could result in the formation of lanthionine (Horn *et al.*, 1941), and in the production of stable lysyl-alanine interchain bridges and racemization (Rudall, 1968).

The urea 5·5 proteins-0 gave 2 peaks on Sepharose 4B (Fig. 3), indicating exclusion and retardation. However, the urea 5·5 protein-25 gave only one peak on Sepharose 4B (Fig. 3) and none of this protein was excluded. The molecular weight of the retarded proteins (4 determinations) as determined from Fig. 4 averaged 5·0 × 10^4, which was somewhat less than the molecular weight (7 × 10^4) determined by Woernley *et al.* (1957), or of that (6 × 10^4) cited by Rudall (1952). Thus urea at room temperature appeared to be more effective in cleaving high molecular weight epidermal proteins than did urea at 0°C. Nevertheless, as determined by immunodiffusion, both the urea 5·5 proteins-0 (both peaks) and -25 have common antigenic determinants with the more complex epidermal proteins. Electrophoretic and sedimentation analyses of the urea 5·5 proteins-0 indicated that they were fairly homogenous (Carruthers *et al.*, 1955; Woernley *et al.*, 1957), but gel filtration as shown above indicated some heterogeneity in these proteins.

When the urea 5·5 protein-0 was centrifuged at 100,000 × *g* for 2 hours, a gel was obtained which represented about 50% of the total protein. This gel and the supernatant protein solution showed precipitin bands of identity in immunodiffusion in agar with antisera prepared against the urea 5·5 protein-0. Thus one can assume that 6 mol/l urea at 25°C converted this gel to the protein which was completely retarded as shown by Sepharose 4B gel filtration (Fig. 3). The urea 4·5 protein-0 has a molecular weight of 3 × 10^4 (Woernley *et al.*, 1957). On Sepharose 4B the urea 4·5 protein-0 was largely retarded to form 2 protein peaks with molecular weights of 3·6 × 10^4 and 1·5 × 10^4. Previous sedimentation (Woernley *et al.*, 1957) and electrophoretic data (Carruthers *et al.*, 1955) had indicated that the urea 4·5 protein-0 was not monodisperse.

The urea 4·5 and 5·5 proteins are related immunochemically, and both of these proteins may be incorporated into the keratin macromolecule (Rudall, 1952). Bernstein *et al.* (1970) extracted a histidine-rich protein from newborn rat and human epidermis. This protein also precipitated at pH 4·5 and has a molecular weight of 3 × 10^4. Synthesis of this histidine-rich protein occurs in the cells of the granular layer, and, according to Bernstein *et al.* (1970), may play a role in the biogenesis of keratohyalin.

Although the axial ratios for several of the urea- and alkali-extracted proteins of beef snout epidermis are rather high (Woernley *et al.*, 1957), the molecular weights obtained by gel filtration agree fairly well with those obtained by classical procedures. Asymmetric protein molecules may be retarded in gel filtration (Andrews, 1970).

With upward flow chromatography on Sepharose 4B in 6 mol/l urea buffered at pH 8·6 with 0·05 mol/l barbital buffer containing 0·1 mol/l NaCl, the urea 6·3 proteins showed only 2 peaks, one of which was excluded, and one of which was retarded (Fig. 5). The proteins from the 2 peak tubes of the urea 6·3 proteins were dialysed against distilled water at pH 9, and then tested by immunodiffusion together with the urea 5·5 protein-0, urea 6·3 proteins, alkali-soluble 5·5 protein and urea 4·5 proteins-25 against antisera raised against the urea 5·5-0 and 6·3 proteins. Two precipitin bands formed and they showed identity with the

6 antigens. These results again indicate possible common antigenicity between the soluble and insoluble beef snout epidermal proteins and their derivatives. The proteins of the retarded peak of the urea 6·3 proteins have an approximate molecular weight of $2{\cdot}7 \times 10^5$ (calibration curve not shown).

Chromatography on Sepharose 4B of the *S*-carboxymethyl derivatives of the urea 6·3 proteins in 6 mol/l urea buffered with 0·05 mol/l barbital at pH 8·6 and containing 0·1 mol/l NaCl showed retardation. These *S*-carboxymethyl derivatives have a molecular weight of approximately $2{\cdot}2 \times 10^5$.

The lyophilized urea 6·3 protein was 88% retarded on Sepharose 4B in 6 mol/l urea alone in contrast to the urea 6·3 proteins, part of which was excluded (50%) and part retarded (50%). This difference was probably due to the fact that, upon dialysis of the tris buffered 6 mol/l urea solution of the urea 6·3 proteins against distilled water at pH 9·0, insoluble proteins gradually came out of solution. These proteins would appear to be those giving rise to peak 2 (Fig. 5). Actually, the urea 6·3 proteins did not remain completely in solution on standing at 0°C in 0·05 mol/l barbital buffer, pH 8·6 unless urea was present.

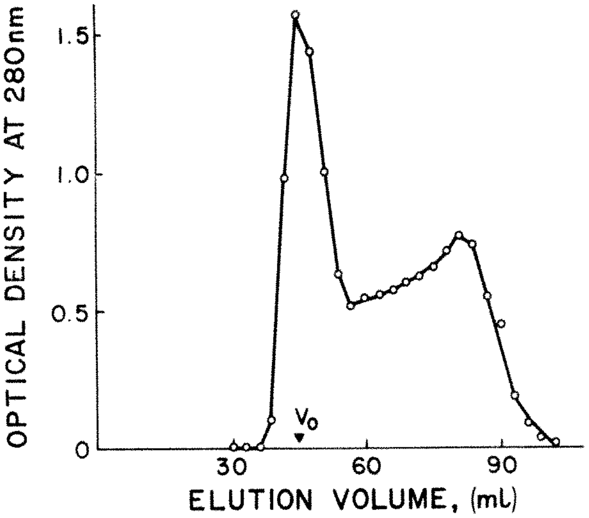

FIG. 5.—Elution profile by upward flow chromatography of Sepharose 4B (bed dimensions 2·5 cm × 28·5 cm) of the urea 6·3 proteins. V_0, the void volume, was 45 ml and the flow rate was 13 ml/hour. These proteins were dialysed overnight against 0·05 mol/l barbital buffer, pH 8·6, containing 0·1 mol/l NaCl and 6 mol/l urea; the same solution was used to elute the proteins.

Amino-acid analyses

A comparison of the amino-acid analyses of the epidermal proteins is given in Table I. There appeared to be no significant differences in the amino-acid composition (except the cystine content) of peak 1 and 2 proteins obtained by chromatography of the urea 6·3 proteins. This observation agreed with their similar behaviour in double diffusion in agar with antisera prepared against the urea 6·3 proteins. The amino-acid analysis of the alkali-soluble 5·5 protein showed slightly less serine and glycine, but more proline and alanine than the urea 6·3 proteins. Cystine is absent from the alkali-soluble 5·5 protein due to the formation of lanthionine, β-amino-β-carboxyethyl sulphide (Horn *et al.*, 1941). The urea 5·5-0 and -25 proteins had a somewhat similar amino-acid composition as well as producing precipitin bands of identity in double diffusion in agar with antisera prepared against the urea 5·5 protein-0. These urea 5·5-0 and -25 proteins have less serine, glycine, and arginine, but somewhat more aspartic acid, proline, and lysine than the urea 6·3 proteins, but the significance of these differences is not known. All of these proteins have relatively low levels of cystine and methionine. The lyophilized urea 6·3 protein contained slightly more proline and glycine than did the urea 6·3 proteins (peak 1), but otherwise the amino-acid composition was quite similar.

TABLE I.—*Amino-acid Composition of Various Epidermal Proteins**

Amino-acid	Urea 5·5 proteins 0	Urea 5·5 proteins 25	Alkali-soluble 5·5 protein	Lyophilized urea 6·3 protein	Proteins from chromatography of urea 6·3 proteins Peaks 1	Proteins from chromatography of urea 6·3 proteins Peaks 2	Matoltsy's pre-keratin†	Pre-keratin Baden‡
Aspartic	9·60	9·81	8·21	8·72	8·69	8·81	8·52	9·1
Threonine	4·74	5·36	3·88	4·06	4·18	3·73	3·66	4·0
Serine	7·64	7·00	8·91	9·83	9·77	10·06	10·85	11·1
Glutamic	13·78	14·43	13·50	13·23	13·96	14·07	16·09	14·1
Proline	3·78	4·83	2·50	2·40	1·72	1·83	1·51	1·4
Glycine	10·67	9·93	13·71	17·04	15·64	16·55	14·06	16·4
Alanine	8·22	7·44	8·91	7·01	7·19	7·61	5·90	6·7
Valine	6·10	6·20	5·60	5·03	5·21	4·69	4·76	4·0
Half cystine	0·87	1·09	0	0·91	0·89	0·40	0·77	0·6
Methionine	1·94	1·15	1·97	1·46	1·18	1·49	0·22	1·3
Isoleucine	4·35	4·07	4·24	3·78	4·27	3·87	3·93	3·5
Leucine	8·98	8·68	9·50	8·22	8·87	9·03	8·50	9·2
Tyrosine	2·72	2·68	2·91	2·85	2·87	2·74	2·19	2·8
Phenylalanine	3·63	3·48	3·85	3·50	3·52	3·61	3·74	3·6
Lysine	6·18	7·02	5·18	5·19	5·20	4·73	6·36	5·1
Histidine	1·62	1·89	1·04	1·06	1·00	0·72	1·16	1·0
Arginine	5·10	4·87	6·02	5·61	5·77	6·03	7·25	6·1

* Results expressed as residues per 100 residues.
† Calculated from Matoltsy (1965).
‡ From Baden *et al.* (1971).

The epidermal alkali-extracted 6·3 proteins are considered by Crounse (1963) to be the most likely candidates for the keratin macromolecules. Crounse extracted with dilute NaOH from human callus, stratum corneum and epidermis proteins which precipitated maximally at pH 6·3, 5·5, 5·0 and 4·5. Amino-acid analyses of the various proteins were determined, the results of which suggested that the 5·0 and 4·5 proteins represent intermediate stages in the formation of the 6·3 epidermal proteins. Since the urea 6·3 proteins appear to be cleaved to yield the urea 4·5 and 5·5 proteins (Carruthers *et al.*, 1955); and, since (as reported here) the urea 4·5, 5·5 and 6·3 proteins have common antigenic determinants, Crounse's hypothesis appears reasonable. Rudall (1968) has discussed some aspects of the relationships between mammalian epidermal proteins.

Matoltsy (1964) has extracted a prekeratin from beef snout epidermis with a 0·1 mol/l citric acid-sodium citrate buffer, pH 2·6. This prekeratin was monodisperse as measured by moving boundary electrophoresis, and by sedimentation analysis. Matoltsy's prekeratin has a molecular weight of 640,000, a particle length of 105 nm, and a diameter of 3·7 nm. The helix content is given as about 40%. Fibres prepared from the prekeratin formed a typical alpha-pattern, with a meridional arc at 0·514 nm and an equatorial arc at 0·975 nm. It is not known how this prekeratin is related to the urea or alkali 6·3 protein macromolecules. The amino-acid analysis of the prekeratin of Matoltsy (1965) is compared with that of the urea 6·3 proteins in Table I. The agreement in the amino-acid composition is quite good except that Matoltsy's prekeratin has more glutamic acid, arginine, serine, and lysine, but much less methionine than does the urea 6·3 protein of peak 1 in Fig. 5. Although several tubes containing the greatest concentration of proteins were used for the amino-acid analyses of the urea 6·3 proteins (Fig. 5 and Table I), no evidence is available that these protein solutions were homogenous. Hence, any agreement in amino-acid composition between the urea 6·3 proteins and the prekeratin of Matoltsy may be fortuitous.

Baden *et al.* (1971) have isolated a prekeratin of beef snout epidermis by the citrate-buffer procedure of Matoltsy, and also a precursor pool of α-fibrous protein from the microsomal fraction of epidermal homogenates. The amino-acid composition and x-ray diffraction patterns of the α-fibrous protein and its precursor were similar. There were some differences, however, in the amino-acid composition of the prekeratin isolated by Baden *et al.* (1971) and by Matoltsy (1965). Yet, in general, there was a noticeable similarity in the amino-acid composition of the prekeratin of Baden *et al.*, Matoltsy, and that given by the peak 1 and 2 urea 6·3 proteins (Table I).

The somewhat similar amino-acid composition of the proteins extracted from beef snout epidermis with urea and alkali suggests that these proteins have common sub-units, but the proteins differ in their degree of polymerization of these protein sub-units. This hypothesis is fortified by the immunodiffusion data which demonstrated that the low molecular weight urea 4·5 and 5·5 proteins had antigenic determinants in common with the high molecular weight urea 6·3 and alkali 5·5 proteins. In this connection Bauer (1971) has demonstrated that the alkali-soluble protein (pH 5·5 fraction) of human epidermis, stratum corneum or callus can occur in various states of aggregation, and that the alkali-soluble proteins had mostly retained their native structures. Bauer also showed that the pH 4·5, 5·0 and 5·5 alkali-soluble proteins of human epidermis were immunochemically identical. Thus, in human and beef snout epidermis more evidence accumulates that the keratin macromolecules are probably formed by polymerization of relatively simple and identical protein sub-units, characteristic for each species.

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A COMPARISON OF *IN VIVO* AND *IN VITRO* METHODS OF IDENTIFYING HUMAN EPIDERMAL CELLS IN DNA SYNTHESIS

ELIZABETH HELL AND HOWARD MAIBACH

Summary.—For identifying epidermal cells in DNA synthesis there are 2 rather different methods in use: subcutaneous injection of ^{3}H thymidine *in vivo*, and short term incubation of skin slices with ^{3}H thymidine *in vitro*.

A direct comparison of these methods on the skin of the same individuals at the same time shows little difference in the results obtained. If anything, the *in vivo* method is slightly more sensitive.

THE technique of identifying cells in DNA synthesis by short-term exposure to ^{3}H thymidine and subsequent production of autoradiographs has been widely used. Inevitably, however, methods vary from one laboratory to another so that strict comparison between the work of different groups is sometimes hazardous. The series of experiments to be described was designed to sort out some of the discrepancies involved.

METHODS

The same healthy human subjects were used for both series of experiments at the same time. In each case there is, therefore, a direct comparison of methods. A piece of skin was excised from one side of a man's back under local anaesthesia (1% lignocaine), using a modification of the technique of Arthur and Shelley (1959). The specimens consisted of epidermis and upper dermis. They were floated dermis side down on a medium of Hanks' balanced salts solution and bovine serum 7 : 3 to which had been added tritiated thymidine 0·7 μCi/ml (360 Ci/mole) After 4 hours' incubation, they were fixed and processed as described by Hell and Cruickshank (1963). The *in vivo* sample was prepared by the intradermal injection of 5 μCi of tritiated thymidine in 0·1 ml of (360 Ci/mole) to the other side of the man's back at the same time as skin for the *in vitro* sample was excised. After 40 min the *in vivo* sample was removed and fixed in buffered formol saline to correspond with the *in vitro* sample (Epstein and Maibach, 1965).

Autoradiographs were prepared from both samples at the same time, exposed, developed, stained and counted in each case as one experiment. Kodak AR10 stripping plates were used, exposure was for 3 days and 7 days and the autoradiographs were processed as described by Hell and Cruickshank (1963).

RESULTS

At least 5000 " viable " epidermal cells were counted from each sample. The proportions of "positive " cells, those having more than twice the background number of black spots located above their nuclei, were calculated in each case. The averages for the 3-day exposure and 7-day exposure are contained in Table I.

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TABLE I.—*Average Percentage Positive Epidermal Cells per Specimen after 3 and 7 Days' Exposure*

	After 3 days		After 7 days	
Exp. no.	*In vivo*	*In vitro*	*In vivo*	*In vitro*
1	1·0	1·0	1·2	1·6
2	1·1	1·0	2·2	1·1
3	3·0	1·1	4·5	1·2
4	2·0	0·5	3·6	0·8
5	1·3	0·9	2·0	1·3
6	1·3	1·3	1·5	2·2
Average	1·62 ± 0·7	0·97 ± 0·24	2·5 ± 1·17	1·37 ± 0·44

$P = 0{\cdot}07$ probability of both being the same, i.e. 93% chance that they are different.

DISCUSSION

The difference between the *in vivo* and *in vitro* results are small in 4 subjects and large in 2. In both cases where there is an appreciable difference, however, this could be due to error. For uninjured normal human skin, 3% positive is rather high; for 13 normal humans the average obtained was 0·9 ± 0·6% (see Hell and Hodgson, 1967). It is possible that the *in vivo* skin sample of Case 3 was the site of a recently healed micro-wound. In Case 4 the *in vitro* sample was incomplete, much of the basal layer being absent. Hence the figure obtained, 0·5%, is probably too low, since many of the positive epidermal cells in uninjured skin are in this layer.

If the 2 groups are averaged without removing the discrepant pairs, the difference between the *in vivo* method and the *in vitro* method is of the order of 70%, which is rather large, although not significant. Systematic false negatives on the autoradiographs of the *in vitro* specimens would be the most likely explanation for a discrepancy of this order. However, the longer period of exposure of the autoradiographs did not remove this: hence there may be a slight but real diminution in pick up of cells in DNA synthesis if they are identified by *in vitro* labelling.

It might have been considered preferable to use the same amount of tritiated thymidine *in vitro* and *in vivo*, and also the same amount of time. However, the methods being compared have each been used for other experiments; it was therefore considered more useful to compare them without alterations, and this is a valid comparison of 2 standard techniques.

A similar series of experiments done elsewhere are reported to have shown no difference between pick up *in vivo* and *in vitro* (Lachapelle, 1969).

We should like to thank Dr C. N. D. Cruickshank for helpful discussion of these results.

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THE QUANTITATION OF KERATINIZATION IN HUMAN FOETAL TISSUES

A. JARRETT AND W. VAN WYK*

Summary.—A method is described for the estimation of disulphide bonds in keratinizing structures by determining the intensity of yellow fluorescence with thioflavine T following oxidation with peracetic acid. Because nuclear proteins fluoresce with thioflavine T, these were removed by RNase and DNase digestion prior to oxidation. Foetal human tissues were examined, and it was found that the hair contained the greatest number of disulphide groupings; both inner root sheath and skin keratins showed definite evidence of disulphide bonding, but there was no evidence that the superficial layer of the palate had disulphide links.

THE most characteristic feature of keratinized structures is the presence of disulphide bonds. These are formed by the oxidation of 2 cysteine residues contained in adjacent polypeptide chains to form a cystine molecule which unites the chains together by 2 sulphur atoms. If the number of these linkages could be determined in a keratinizing structure, it would give a function of the degree of keratinization as expressed by disulphide formation.

When disulphide bonds are oxidized by peracetic acid to cysteic acid, the latter can be linked with thioflavine T to give a complex which fluoresces brilliantly when irradiated with u.v. rays (Jarrett *et al.*, 1959). If the intensity of the induced fluorescence is proportional to the number of disulphide cross-linkages in the original structure, a méasurement of the induced fluorescence would be related to the number of disulphide bonds and thus to the degree of keratinization.

Dr Gillespie kindly supplied samples of wool whose cystine content had been estimated by chemical means; these were subjected to peracetic acid oxidation and fluorochromed with thioflavine T. A reasonable correlation between the disulphide content and the intensity of the fluorescence was obtained, despite gross variation in the fluorescence of individual fibres. Furthermore, it was found possible to obtain a satisfactory fluorescence using blue light in the region of 550 nm instead of u.v. rays having a wave length of about 360 nm.

It has also been shown (Jarrett, 1963) that thioflavine T fluoresces a brilliant yellow with both ribonucleic and deoxyribonucleic acids. These would obviously cause interference with the measurement of the cysteic acid formed by the oxidation of disulphide linkages. It was therefore necessary to remove these compounds with ribonuclease and deoxyribonuclease prior to oxidation with peracetic acid. This is most important with respect to inner root sheath keratin, as it has been shown that this tissue contains a significant amount of ribonucleic acid and therefore fluoresces yellow without pretreatment with peracetic acid (Jarrett, 1958).

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MATERIALS AND METHODS

Material from a human foetus with a crown-rump length of 175 mm was fixed in Bouin's fluid, embedded in paraffin, and sectioned in the usual manner. Sections were taken to water, and then treated with a mixture of RNase and DNase, each in a concentration of 0·4% buffered to pH 6·5 with phosphate buffer for a period of 1 hour at 37 °C. They were then washed and treated with 30% peracetic acid at room temperature for 30 min and stained with 0·1% thioflavine T at pH 4·5 for 5 min.

The degree of yellow fluorescence was measured by a highly sensitive Reichert photomultiplier fitted with a step wedge interference filter which enabled the fluorescence to be measured at a series of wavelengths and thus establish its spectral characteristics. The exciting radiation was provided by a quartz-iodine lamp and passed through a blue filter having a maximum transmission at 550 nm. An orange filter was placed above the fluorescing specimen to remove extraneous light and to give a black background to the yellow fluorescence.

It was thought that this orange filter might modify the spectral characteristics of the yellow fluorescence of the cysteic acid-thioflavine T complex. To test this, an HBO 200 high pressure mercury vapour light source was substituted for the quartz iodine lamp, and filters having a transmission in the region of 360 nm were used to excite the fluorescence. A Wratten 2B filter was used to replace the orange filter and the spectral curve of the fluorescing emission checked against that obtained with the previous light source and filter system. No significant difference was found between the curves obtained by either method. It was therefore decided to use the quartz iodine lamp because it gave a more constant output of light; and, as it was run at 13 V, its supply could be more readily stabilized than that for the HBO 200. All the results reported in this paper were therefore obtained with blue fluorescence using a quartz iodine light source.

A number of different types of foetal epidermis were examined from the same section of foetus. It is essential to use the same section for comparative purposes because, under these circumstances, all the tissues had been exposed to the same degree of DNase and RNase digestion and to the same intensity of peracetic acid oxidation. The sites examined included the skin of the lip, the hard palate, the mucous membrane of the lip, the hair shafts from the developing follicle on the face, and the inner root sheath keratin from the same follicles. Three series of readings were taken from each of these areas, and the average results are shown in Table I. In addition, a blank reading was obtained from the light passing through the filter system by interposing non-fluorescing tissue from the same section: these control readings are shown in the table.

RESULTS

The intensity of the yellow fluorescence was measured at a series of wavelengths ranging from 500 nm to 680 nm. The average of 3 results obtained at different sites are given in Table I. It will be readily seen that the hair shaft gave the strongest fluorescence, and this is indicative of a greater number of disulphide bonds. Inner root sheath keratin gave the second highest readings; it is unlikely that this is due to contained nuclear proteins, as these were removed by pre-treatment with DNase and RNase. Epidermis from the lip showed definite evidence of disulphide bonding, but that of the hard palate was not significantly different from that of the unkeratinized oral mucosa (Table I; Fig. 1). The latter gave virtually the same values of fluorescence intensity as the control values obtained from the light passing through the filter system. Therefore there was no definite evidence that keratinization had occurred either in the hard palate epithelium or in the oral mucosa.

TABLE I.—*Average of 3 Readings at Different Sites on the Structure Examined*

Wavelength (nm)	Hair keratin	Inner root sheath keratin	Skin keratin	Palate keratin	Non-keratinized labial mucosa	Control
500	0	0	0	0	0·3	0
520	10·3	5·2	4·8	3·0	2·8	2·5
540	39·0	16·0	12·0	4·1	3·0	2·8
550	53·0	15·5	12·5	6·4	4·0	3·8
560	53·0	16·2	15·0	8·6	6·3	6·0
580	33·0	6·8	5·5	2·7	1·8	1·5
600	14·2	2·7	1·8	0·7	0·3	0
610	7·5	1·7	1·0	0·3	0	0
620	5·0	0·9	0·5	0	0	0
650	0·7	0	0	0	0	0
680	0	0	0	0	0	0

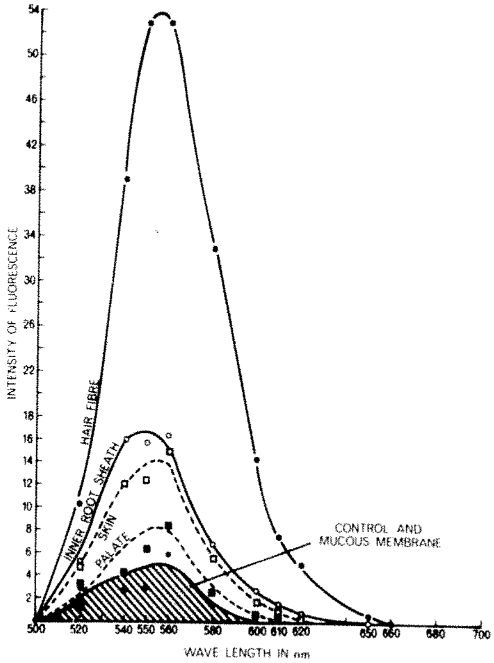

FIG. 1.—Comparison of the intensities of fluorescence of various epidermal structures.

The ratios between the intensity of the hair fluorescence and that of the inner root sheath keratin, the skin and the palate were determined at all the wavelengths examined. These were calculated by subtracting the log of the fluorescence intensity of the structure at a particular wavelength from the log of the fluorescence intensity, at the same wavelength, of the hair shaft. The results of the ratios with respect to hair/inner root sheath and hair/palate are shown in Figs 2, 3. It will be seen that the ratio varies according to the wavelength and that there is a tendency for this ratio to be relatively constant where the wave flattens at the region of maximum fluorescence intensity, that is, in the wavelength range 540–560 nm.

This finding indicates that the comparison of the fluorescence was most accurate when made at the wavelength of maximum fluorescence intensity. Thus, readings made at 550 nm, under standard conditions, could be compared directly one with another without the necessity of obtaining a full spectral curve.

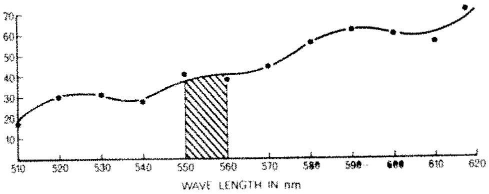

Fig. 2.—Ratio of hair readings (Exper. 1) to inner root sheath keratin (Exper. 1). (Log intensity hair reading–log intensity inner root sheath reading.)

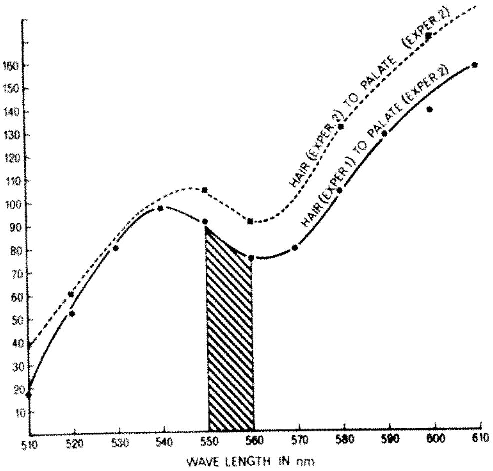

Fig. 3.—Ratio of hair readings to palate readings. (Log intensity hair reading–log intensity palate readings.)

DISCUSSION

The results reported in this paper show clearly that the hair fibre has a far greater intensity of fluorescence than any other epidermal structure examined. The inner root sheath, which undergoes a different mode of keratinization (Jarrett, 1958), shows the second greatest fluorescence. The keratin layer of lip skin shows relatively little fluorescence, and this is taken to indicate that it contains relatively few disulphide bonds. Also, the palate shows virtually no evidence of the formation of disulphide linkage and gives a similar intensity to that of the labial mucosa, which never becomes keratinized—even in the adult. It is usually accepted that the labial and buccal mucosa normally never become keratinized, but the hard palate does form a thin layer of keratin.

Bowman and Latham (1968) claimed to have demonstrated the presence of disulphide groups in the palatal mucosa of late foetal life. However, they used the dihydroxy–dinaphthyl disulphide method, and this is notoriously difficult, as the disulphide is demonstrated as sulphydryl after blocking the sulphydryl groups already present and then reducing the disulphide to sulphydryl. It will be appreciated that false positives are likely to occur when using this technique, as some of the original sulphydryl groups may escape the blocking agent or be re-exposed by the subsequent reduction.

It would certainly appear that, at the age of this foetus, there was no evidence of keratinization; but it remains to be shown whether keratinization, as defined by the above technique, can be demonstrated—even in the adult. The question of keratinization of the mucous membranes of the mouth and vagina must be studied further in order to establish whether at any time they produce a true keratin containing disulphide linkages. The vagina, and perhaps also the mouth, undergoes cyclical changes during the menstrual cycle, but whether the so-called cornified phase keratin actually produces keratin which contains disulphide bonds is a matter for further investigation.

The present study is comparative; before absolute values for the cystine content of keratinized structure can be made by this technique, it will be necessary to establish the relationship between the intensity of the fluorescence and the cystine content of previously analysed hair samples. It would then be possible to calibrate the equipment so that, under standard conditions, direct estimations could be made of the disulphide content of keratinized structures. A most difficult part of such a procedure would be the standardization of the degree of peracetic oxidation, as this acid is notoriously unstable and loses its activity on standing. However, if acid of a sufficient strength is used, then maximum oxidation should be achieved. At present, the method has been used as a comparative technique between tissues which have been simultaneously oxidized by a given sample of peracetic acid.

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WHITE SCALP HAIRS TURNING BLACK—AN UNUSUAL REVERSAL OF THE AGEING PROCESS

STANLEY COMAISH

DURING the course of a study on factors affecting hair growth, an individual was encountered showing what appears to be a most unusual feature. The subject is a healthy Caucasoid male aged 38, with no clinical abnormality of the scalp or skin, apart from some solar changes in the exposed skin of the temples. He has never had alopecia areata or any other hair abnormality, apart from the usual ageing changes of frontal recession. He does not dye or tint the hair. Like his father, he tended to become grey early in life; some grey hairs were visible at the sides of the scalp from the age of 17 years, and there is now considerable greying of the hair shafts in these areas and posteriorly. The vast majority of these hairs are either all white or all black, but 3 hairs plucked from his scalp on separate occasions show the remarkable feature of loss of pigment distally with normally pigmented proximal parts and roots (Fig. 1). Both naked eye and microscopical examination confirm that these roots are normal anagen hairs.

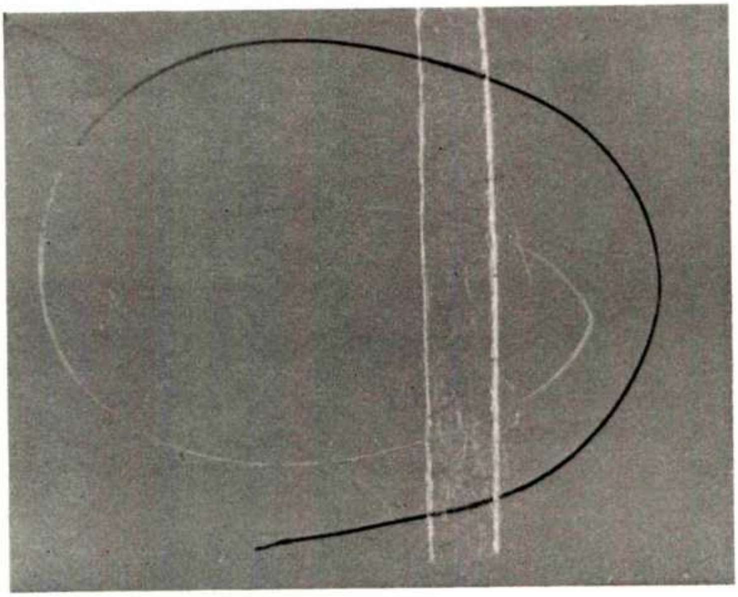

FIG. 1.—Hair showing loss of pigment distally with recovery of normal pigmentation in lower shaft.

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and the hair shafts themselves appear to be quite normal. The transition from black to white is a gradual one, occurring over a length of 5–8 mm. Scanning electron microscopy of one of these hairs discloses a perfectly normal cuticular pattern (Fig. 2), with the increasing crenellation of the cuticle edges and dystrophy from base to tip along the shaft associated with ordinary wear-and-tear (Bottoms *et al.*, 1972). These 3 hairs are quite unrepresentative, and the head hair as a whole continues to grow steadily more grey with advancing years.

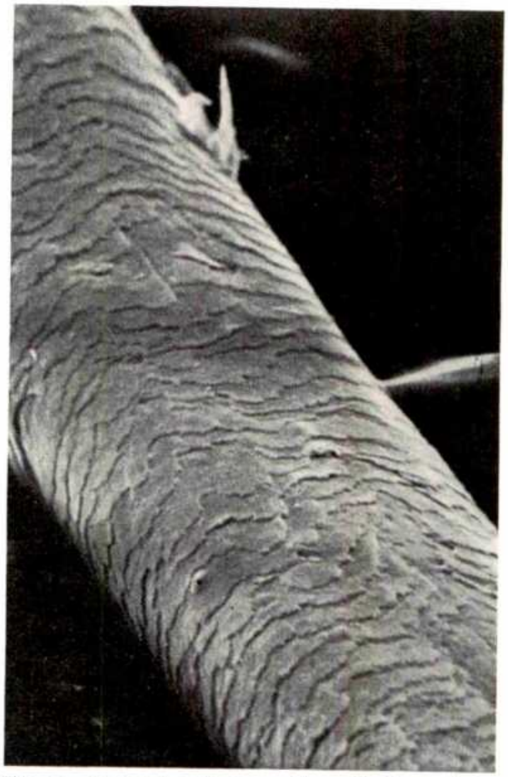

FIG. 2.—Normal cuticular pattern of affected hair.

There are few examples in the literature of white or grey hair becoming repigmented, other than following alopecia areata or severe systemic illness, under which circumstances it is the growth of new pigmented hairs rather than the repigmentation of a growing shaft. Naegeli (1933), however, has reported observations similar to my own, and Savill and Warren (1962) review the whole subject of repigmentation of grey and white hairs. In this connection it is interesting to note that greying of hair was found to be the physical sign most closely correlating with chronological age of 9 physiological functions examined by theAtomic Bomb Casualty Commission, Hiroshima (Hollingsworth *et al.*, 1965).

For completeness sake I should record that the other 8 parameters of ageing in my patient are progressing in their usual orderly fashion and in the orthodox direction, towards senescence.

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Br. J. Derm. (1972) **86**, 515

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CONGENITAL DIFFUSE MOTTLING OF THE SKIN

D. V. STEVANOVIĆ

Summary.—Unusual mottled pigmentation present since birth in a girl aged 16 is described. Its pattern seems to differ from any previously reported. Through 3 generations in the direct ascending line generalized lentiginosis has been present. The hereditary background of pigmentary changes in the girl is discussed.

A GIRL with unusual mottled pigmentation of the skin and buccal mucosa is reported. The pattern of lesions seems not to have been previously reported.

CASE REPORT

Lj.K., a schoolgirl aged 16 first consulted us in 1958; she has since been seen on several other occasions at irregular intervals.

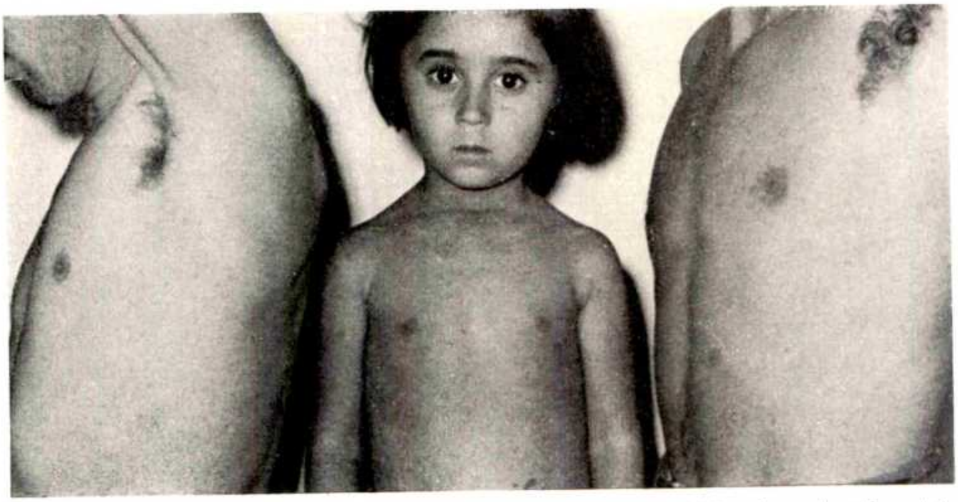

FIG. 1.—The pigmentary changes in the child, her father and his half-brother. A café-au-lait spot can be seen on the abdomen in the half-brother (photograph taken in 1958).

Her father (M.K.) stated in 1958 that the unusual pigmentary changes in his daughter have been present since birth. He further added that, as well as himself, his half-brother (R.K.) (Fig. 1) (their father was married twice) their father (L.K.) and their grandfather, now dead, also had similar skin changes present since birth.

The whole family, living not far from Belgrade, was then examined. In the course of the preparation of this paper all members were re-examined (Table I). In 1963 and 1965 2 children were born to the affected half-brother (R.K.).

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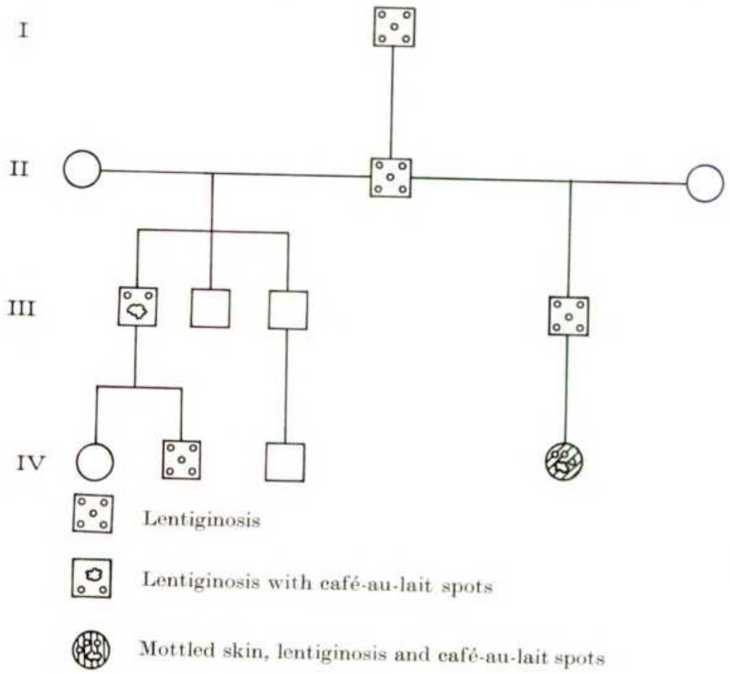
TABLE I.—*The Family Pedigree with 4 Generations of Affected Members*


I
II
III
IV
Lentiginosis
Lentiginosis with café-au-lait spots
Mottled skin, lentiginosis and café-au-lait spots

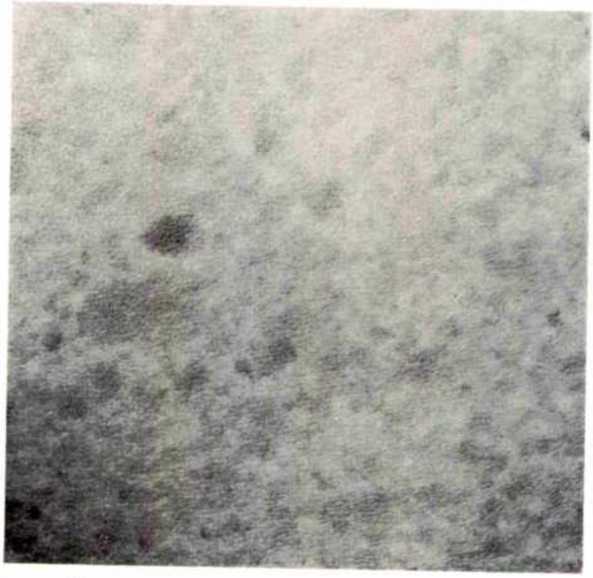


FIG. 2.—Close-up of the pigmentary changes in the child (photograph taken in 1970).

When she was examined in 1958 the child's entire skin, the face excepted, exhibited a mottled pigmentation. The mottling consisted of light and dark brown macules; they were of irregular shape and of various sizes, up to 2 cm in diameter. Among the numerous macules were small areas of normal skin. True depigmentation was not present (Fig. 2). In addition there were many café-au-lait spots scattered over the whole body (Fig. 3).

Irregularly shaped, pigmented patches were present also on the palms, but not on the soles. The proximal part of the nailbeds of all fingers was slightly pigmented (Fig. 4). On the face and lips in a random distribution, neither periorificially nor centrofacially, several small round intensely pigmented macules were present. Several similar lesions were seen over buccal mucosa and on the gums. In the latter site the pigmentation was band-like (Fig. 5). General examination revealed no other abnormality.

Comparing the photographs taken in 1958 (Fig. 1) with those of later dates (Fig. 2 to 5), there was only some relative enlargement of the macules. Their number, intensity of colour and pattern remained unchanged.

Each summer the patient exposed herself with impunity to the sun. Her whole skin became darker, pigmented macules included.

Routine laboratory tests, several times repeated, were within the limits of normal. ECG normal. X-ray examination showed no gastrointestinal or skeletal abnormalities.

Two biopsies of pigmented macules (in 1958 and 1970) have been carried out.

The histological findings in the sections from both biopsies were essentially the same. The rete ridges were elongated, often club-shaped. The basal layer showed considerable hyperpigmentation, with an increase in clear cells. In the upper dermis there was a mild perivascular infiltrate composed of lymphocytes and histiocytes (Fig. 6).

The patient's (Lj.K.) grandfather (L.K.) and father (M.K.) have macular lesions brownish in colour, distributed over the whole of the body, with the exception of the face. The appearance is that of lentiginosis. The half-brother (R.K.) of the latter (M.K.) has also three café-au-lait spots on his trunk. His son (J.K.) has only macular lesions similar to those of his uncle (M.K.).

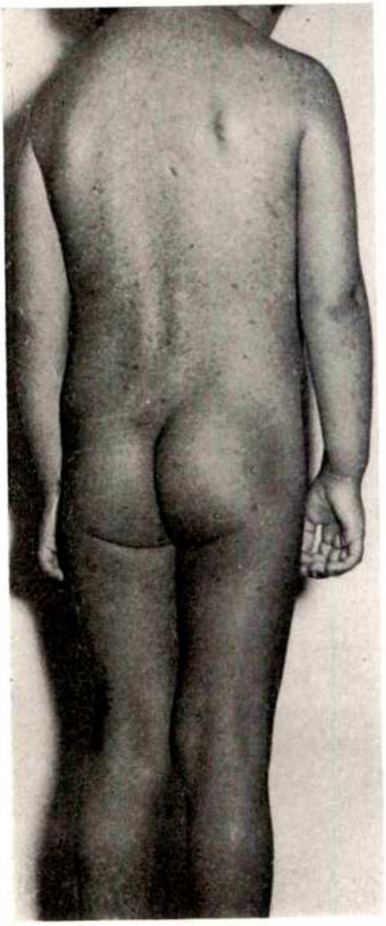


FIG. 3.—The appearance of the back. The scar of a previous biopsy is also seen (photograph taken in 1959).

In late 1958 skin biopsies of pigmented macules were also carried out in M.K. and R.K. The histological findings were essentially the same as those observed in Lj.K.

In 1971 electrocardiography was also carried out in the patient's father (M.K.) and uncle (R.K.) with normal results.

DISCUSSION

Generalized lentiginosis as a dominant autosomal trait is one component of the complex and somewhat variable L.E.O.P.A.R.D. syndrome (Gorlin *et al*., 1969).

but in neither our patient nor her affected relatives were any of the other characteristics of the syndrome detected. There were no electrocardiographic abnormalities. Generalized lentiginosis as an apparently isolated hereditary abnormality has also been reported (Touraine, 1955). However, the sudden appearance of a clinically distinct pigmentary anomaly in the fourth generation in our pedigree with hereditary lentiginosis is unusual.

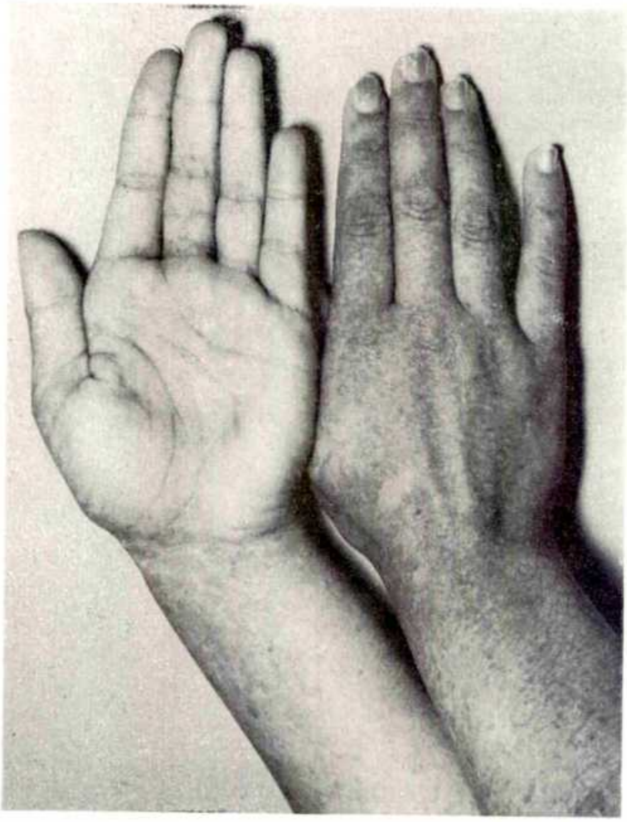


FIG. 4.—The pigmentation of the nailbeds, palms and dorsal aspects of the hands (photograph taken in 1970).

While somewhat resembling lentigines, the patches differed by their unequal size, shape and intensity of colour. The pigmented macules of the oral mucosa and palms recalled the Peutz–Jeghers syndrome. Moreover, there were also *naevi spili*—" café-au-lait spots "—found in some other hereditary conditions of which the pigmentary disorder is not the salient feature (von Recklinghausen's, Albright's and Gaucher's diseases).

The pattern of pigment distribution in our patient closely resembles those reported by Weary *et al.* (1969). The affected patients came from 2 Negro families. Sclerosis of the palms and soles, sclerotic bands in the axillae, the antecubital and popliteal fossae, clubbing of the fingers and calcinosis, were associated features.

Dyschromatosis universalis hereditaria of Toyama–Ichikawa–Hiraga, considered to be related to xeroderma pigmentosum, is a melanoleukoderma (Kitamura, 1962), which appears to be distinctive.

Poikilodermatous changes present from birth, and apparently of dominant inheritance, were seen in Wodniansky's (1957) patient. However, the pigmentary changes were not so widespread, and papillomatosis of the anal area was an associated feature.

Naegeli's "Familiarer Chromatophorennaevus", better known in later publications as "reticular pigmented dermatosis of Franceschetti–Jadassohn" (Curth, 1966) or Naegeli's reticular pigmented dermatosis (Whiting, 1971), is characterized by a basically reticulate and evenly distributed pigmentation, generally appearing at the age of 2 and receding with increasing age, discomfort provoked by heat—owing to the impaired sweat gland function—and a moderate palmo-plantar keratoderma. The histopathological picture is that of incontinentia pigmenti, with large macrophages packed with pigment in the upper dermis. Its inheritance is determined by an autosomal dominant gene.

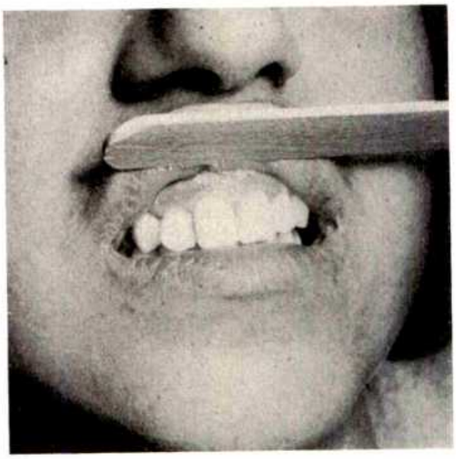


FIG. 5.—Macules are situated on the face and lips; on the gums pigmentation is band-like (photograph taken in 1970).

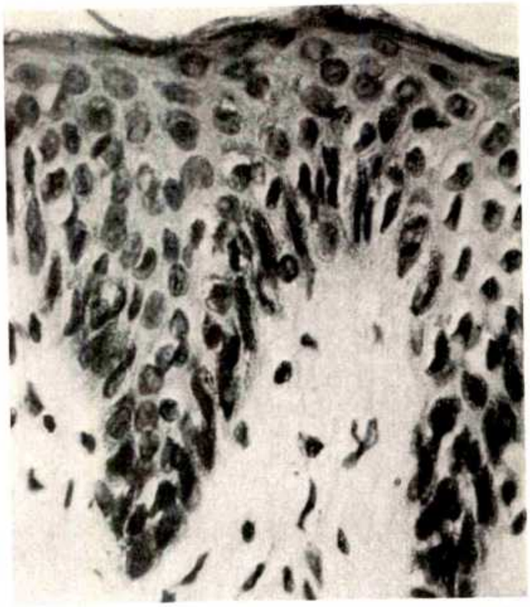


FIG. 6.—The rete ridges are elongated. The basal layer shows considerable pigmentation, with an increase in clear cells.

Apart from the pigmentation, the skin in our patient is completely normal. The lack of associated changes, as well as the distribution of the pigmentation, exclude dyskeratosis congenita, xeroderma pigmentosum and the various poikilodermatous states (Rook *et al.*, 1959).

To what extent pigmented naevi are inherited and the possible modes of inheritance are uncertain. More than one gene may be responsible for the same type of naevus in different pedigrees (Curth, 1966); and, where a single gene appears to be involved, its expressivity is variable. On the basis of our family, in which only one individual showed an apparently unique association of pigmentary abnormalities, it would be unwise to propose any genetic interpretation of the findings.

This paper was supported in part by a grant from the Association of Scientific Medical Institutions of Serbia. Photographs were taken at the Photographic Unit, Medical Faculty, Belgrade. Dedicated to Professor M. Milovanović, M.D., on his 70th birthday.

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DAPSONE INDUCED PERIPHERAL NEUROPATHY

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Summary.—A further case of peripheral neuropathy has occurred in the course of dapsone therapy. It was predominantly motor in type, but a sensory element was present. It recovered completely after dapsone withdrawal. Electromyography and nerve conduction studies showed the neuropathy to be axonal in type.

DESPITE the extensive use of dapsone (4,4′-diaminodiphenylsulphone) in the treatment of various skin conditions, only 2 cases have been published outside the context of leprosy in which peripheral neuropathy was temporarily associated with dapsone therapy (Saqueton *et al.*, 1969). An unpublished case has also come to our attention (Hay, 1971).

We wish to record a further case of this association.

CASE HISTORY

On July 6th, 1969, 2 days after delivery of her second child, a previously healthy housewife aged 24 developed a blistering rash. The diagnosis was uncertain, but the possibilities considered initially were erythema multiforme and herpes gestationis. She was treated with prednisone 100 mg daily, and maintained on a smaller dosage of this drug until December 3rd, 1969, when her rash recurred. At this stage it seemed likely that the rash was that of dermatitis herpetiformis, and she was given dapsone in addition to the prednisone she was already taking. She took dapsone 400 mg daily for a few days, but the dose was soon reduced to 200 mg daily, and prednisone was withdrawn. Six weeks after commencing dapsone and when she had received a total dose of 8·6 g, she began to notice clumsiness of the hands. On February 5th, when she was still taking dapsone 200 mg daily, there was slight weakness of opposition of the thumbs. At the end of February the dapsone was stopped because the rash seemed to be under control. However, on March 16th, the rash again relapsed, and she recommenced taking dapsone 200 mg daily. Neurological assessment on this occasion showed a subjective decrease in superficial pain sensation in the hands, which shaded off to normal at mid-forearm on both sides, and a mild decrease in 2-point discrimination of the fingers; but light touch, vibration and position sensation were normal. No sensory abnormalities were detected elsewhere, including the lower limbs. Muscle testing showed moderate weakness of the thenar muscles of both sides, as well as mild bilateral weakness of the interossei, flexor pollicis longus, finger extensors and peronei. The upper limb reflexes were absent, while the lower limb reflexes were brisk and symmetrically equal. The motor nerve conduction velocity of the right median nerve from elbow to wrist was 55 m/second with a terminal latency from a stimulus at the wrist to the onset of the muscle action potential response of 3·5 milliseconds (both within normal range). Electromyographic examination on March 18th, 1970, showed

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clear evidence of denervation affecting the right abductor pollicis brevis, including spontaneous fibrillation potentials and some long duration polyphasic units. There was possible denervation of the left tibialis anterior.

The patient's rash continued to be troublesome when dapsone was stopped, and she therefore continued to take dapsone in sufficient dosage to control her eruption until June 11th, 1970, when the rash disappeared. Electromyographic study carried out on June 29th showed peak to peak median motor nerve conduction velocity in the forearm unchanged, but the compound muscle action potential evoked by a stimulus to the nerve at the elbow was widely dispersed, indicating slowing of some fibres, since the response to the stimulus delivered at the wrist was homogeneous and biphasic. The amplitude of the compound muscle action potential recorded with surface electrodes over the abductor pollicis brevis muscle, evoked by a supramaximal stimulus to the median nerve at the wrist, was substantially reduced (1·08 mV), while the sensory nerve action potential recorded from the second and third fingers in response to the same stimulus was of normal amplitude (31 μV).

Her muscle weakness gradually improved. On review on October 20th, 1971, 16 months after stopping dapsone, full power was present to clinical testing, and electromyography revealed no persisting abnormality.

The serum vitamin B_{12} concentration, glucose tolerance and urinary porphyrins had been normal, and arsenic was not detected in the hair or urine. Peroral jejunal biopsy showed normal small gut mucosa.

DISCUSSION

In retrospect it seems likely that our patient was suffering from herpes gestationis of puerperal onset. A peripheral neuropathy has not been described in this disease, nor, except for Vickers' patient (Vickers, 1970), in other blistering diseases of the skin. The absence of evidence of other common causes of peripheral neuropathy suggests that one of the drugs she was taking was responsible, and as prednisone is not known to produce a neuropathy, dapsone seemed to be the likely cause, especially in view of the time relationships. Our case differs slightly from those previously described (Saqueton *et al.*, 1969) in that she had mild sensory as well as motor neuropathy, and that her changes were predominantly in the upper limbs. She resembles the other cases in that nerve conduction studies suggest that the neuropathy was probably of axonal rather than demyelinating type, and has resolved after stopping dapsone. In the previous cases the neuropathy occurred after 26 g of dapsone over 11 weeks, and 48 g of dapsone over 5 months respectively. Our patient took a total of 35 g over 139 days; neuropathy started when she had taken 8·6 g over 6 weeks.

Dr Hay's case was a man with dermatitis herpetiformis (Hay, 1971) who developed paraesthesiae and numbness of fingers and toes, and weak legs, after taking about 22·5 g of dapsone over 10 months; his neuropathy recovered 3 weeks after dapsone was withdrawn.

Although dapsone is known to be a competitive inhibitor of para-aminobenzoic acid and is present in the nerves of leprosy patients taking dapsone (Poddar and Chatterjee, 1957), we have no suggestions as to how the neuropathy is actually produced; its mechanism might be clarified if it could be studied in an animal model as in the case of vincristine (Bradley, 1970).

Peripheral neuropathy is part of the natural history of leprosy for which disease much of the world's dapsone supply is used. Neuropathy occurring during dapsone therapy in leprosy is common and is attributed to reactional states in peripheral nerves. Nerve biopsy in such cases should distinguish between the cellular infiltration of reactional states and an axonal neuropathy due to dapsone therapy.

Thanks are due to Dr Colin Brown for the nerve conduction studies and to Drs W. G. Bradley, S. G. Browne, D. A. Shaw, Janet Marks and Professor Sam Shuster for advice.

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RETINOIC ACID IN THE TREATMENT OF PSORIASIS

ANGUS MACDONALD AND LIONEL FRY

Summary.—Thirty-three patients with psoriasis applied retinoic acid ointment and betamethasone 17-valerate ointment to lesions on 1 side of their body, and a placebo ointment and identical betamethasone 17-valerate ointment to symmetrical lesions on the opposite side. Objective assessment of the treated areas showed a significant superiority of the retinoic acid and steroid over the placebo and steroid combination. The mode of action of retinoic acid in psoriasis is discussed.

IN 1962 Stuttgen demonstrated that, when applied topically to human skin, the acid radical of vitamin A (retinoic acid) was considerably more potent than the alcohol (retinol) or other forms of the vitamin. Since then retinoic acid has been used by a number of different workers for the treatment of a variety of conditions. Recently, favourable reports have appeared concerning its use in the following conditions: senile keratosis (Stuttgen, 1962); congenital ichthyosiform erythroderma (Thomson and Milne, 1969); Darier's disease (Hesbacher, 1970), lamellar ichthyosis (Mirrer *et al.*, 1970); ichthyosiform dermatoses and psoriasis (Frost and Weinstein, 1969).

We have recently reported on the histological changes which occur when lesions of psoriasis are treated with retinoic acid over a period of 2 weeks (Fry *et al.*, 1970). This study, although of limited extent showed a significantly favourable response in the psoriatic epidermis in terms of keratin formation and reduced mitotic activity. When the study was extended to cover a period of 3 months, it was found that, because of skin irritation, many patients were unable to tolerate the ointment in its original concentration (0·1%). On theoretical grounds it seemed reasonable that the irritant properties of retinoic acid would be counteracted by a topical steroid preparation, and a trial was therefore conducted in which the effects of retinoic acid used in conjunction with a topical steroid preparation (betamethasone 17-valerate ointment) were compared with the steroid on its own.

PATIENTS AND METHODS

Forty patients were studied, of whom 33 completed the trial. All had received no treatment (other than soft paraffin ointment to diminish scaling) for at least 2 weeks prior to the start of the trial. Betamethasone 17-valerate ointment (Betnovate) was applied twice daily to all lesions to be studied. In addition all patients received retinoic acid 0·1% in a special stabilizing base to be applied to lesions on 1 side of the body, and a placebo ointment (consisting of similarly coloured, unmedicated, but otherwise identical

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base) to be applied to lesions on the opposite side. The ointments were in identical tubes marked right and left, and were applied immediately after the betamethasone. Whether the right or left side was treated by retinoic acid or placebo was determined by random allocation, and was unknown to the investigator until the end of the study.

In many patients the lesions on more than 1 pair of sites were studied, i.e. legs, arms, trunk. Patients were given detailed instructions as to the application of the ointments in order to avoid contamination of the active and placebo areas with inappropriate ointment.

Assessment.—The lesions were assessed in terms of erythema, palpability and scale. Each of these features received a score of 0, 1 or 2, giving a typical plaque of active psoriasis a total score of 6 and normal skin 0.

Observations were made at 4, 8 and 12 weeks entirely blind and recorded without comparison to previous scores. In addition to this a subjective assessment of relative efficacy was made by the patients themselves, and expressed simply as the superiority of 1 side over the other.

RESULTS

Objective assessment

A total of 67 paired observations were made in 33 patients; for each observation the sum of the total clinical scores at 4, 8 and 12 weeks (of a total of 18) was compared, right against left. Superiority of 1 side over the other was not accepted unless the difference between the scores was 2 or more. By this method of assessment results could be summarized thus:

retinoic acid + steroid	equal to	placebo + steroid	37 (55%)
retinoic acid	+ steroid superior		25 (37%)
placebo	+ steroid superior		5 (8%)
			67

Applying the chi-square test to the positive results $\chi^2 = 13{\cdot}32$, showing retinoic acid plus steroid is significantly superior to steroid plus placebo ($P < 0{\cdot}001$).

Subjective assessment

From 3 patient/observations

retinoic acid + steroid	equal to	placebo + steroid	15 (45%)
retinoic acid	+ steroid superior		11 (32%)
placebo	+ steroid superior		7 (22%)
			33

Again applying the chi-square test to the positive results, $\chi^2 = 0{\cdot}52$; this is not a significant difference ($P > 0{\cdot}5$).

An additional and striking observation was the wide disparity of response amongst patients treated with retinoic acid. In some the improvement, although detectable, was not obvious, whereas in others it was remarkable, showing complete unilateral clearing of the lesions (Fig. 1). There was no erythema, irritation, or other significant side effect in any of the patients.

DISCUSSION

These results show that, in a significant proportion of patients with psoriasis, retinoic acid ointment plus a topical steroid is superior to the steroid alone when judged by clearing of the lesions.

Vitamin A (retinol) and its derivatives have been widely used in the treatment of skin disease for many years, but results have never been consistent or satisfactory enough to establish its general acceptance.

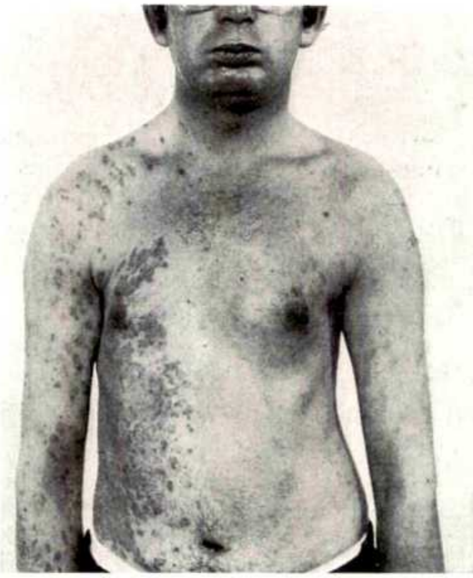

FIG. 1.—Eight weeks after commencement of treatment. Left side of body treated with retinoic acid plus betamethasone shows clearing of psoriasis compared to right side, treated with placebo plus betamethasone, where lesions are still palpable.

In the resurgence of interest which followed Stuttgen's demonstration of the relative potency of topical retinoic acid, psoriasis has received some attention, but formulation of the substance is difficult, and it is only recently that stable preparations have been available for more searching evaluation of their efficacy.

The mode of action of retinoic acid is not entirely straightforward. In psoriasis it has 2, possibly quite independent, actions. First, it has a " normalizing " effect on keratinization, as seen by reformation of the granular layer of the epidermis and loss of parakeratosis (Fry *et al.*, 1970; Frost and Weinstein, 1969). Secondly, it may cause increased epidermal mitotic activity in the basal layer (Kligman *et al.*, 1969). This dual action has been well demonstrated in normal guinea-pig skin by Christophers and Braun-Falco (1970). They showed that repeated application of retinoic acid (0·5% acetone solution) led to a considerable increase in mitotic figures and thickening of the epidermis, but with no parakeratosis, despite an epidermal turnover time of 3 days: in fact, the keratin formed was normal in appearance.

These apparently unrelated actions of retinoic acid account for the many misunderstandings which have arisen regarding its mode of action (*Br. med. J.*, leading article, 1969; Riley, 1969). In psoriasis the beneficial action of retinoic acid on keratinization may be reversed by its irritant effect. It is however possible to control this effect by the simultaneous application of a topical steroid (Frost and Weinstein, 1969; Mirrer and McGuire, 1970).

Although the actual mode of application used in this study is impractical, the need for a cosmetically acceptable preparation, which is more effective than topical steroids on their own, is so great that it would seem reasonable to pursue retinoic acid/steroid combinations to a further stage.

It is perhaps surprising that a similar result obtained using triamcinolone and retinol (Vitamin A) by topical application (Whittle *et al.*, 1961) was not more energetically followed up.

Assuming that the problems of pharmacological stability are overcome, the fact which appears to limit the use of retinoic acid most prominently is the great variation in tolerance and response; this of course applies to other agents used in the treatment of psoriasis, and as with them, variations in the strength of retinoic acid and steroid may prove beneficial. If this is the case, this combination may be of help in the management of this difficult disease.

We are grateful to Dr Neville Shepherd of Ortho Pharmaceuticals for the supplies of retinoic acid ointment, and to Miss L. Wilson of Glaxo Laboratories for the betamethasone 17-valerate ointment used in the study.

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NAEVUS COMEDONICUS—TREATMENT WITH RETINOIC ACID

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Summary.—Two patients are described with naevus comedonicus. Both patients were successfully treated with topical retinoic acid with a minimum of irritation. Continued periodic application of retinoic acid is necessary to prevent reaccumulation of horny material in the involved follicles.

NAEVUS COMEDONICUS is a relatively rare abnormality characterized by groups or bands of large comedones which are present from birth or appear early in life (Wood and Thew, 1968). The typical grouping of comedones, age of onset and persistence easily distinguish this condition from acne vulgaris. Except for isolated reports of association with ichthyosis (Piers, 1945), linear basal cell naevus (Carney, 1952), Sturge Weber syndrome (Rook, 1952), and ipsilateral cataract (Whyte, 1968), naevus comedonicus has presented as an isolated developmental defect. Effective treatment has been limited in the past to manual extraction of the impacted horny material from each involved follicle. This report describes the successful treatment of naevus comedonicus in 2 patients with retinoic acid.

CASE REPORTS

Patient 1.—A Negro male aged 10 presented to the Dermatology Clinic of the Children's Hospital of Philadelphia because of a skin condition which had been present since birth. It consisted of a patch of open and closed comedones, measuring 3 × 4 cm, in the middle of his forehead and extending onto the bridge of his nose. There was also a linear band of comedones extending from the left corner of his mouth on to his cheek. No other cutaneous abnormalities were noted upon close examination. The remainder of the physical examination was normal. Treatment consisted of daily application of 0·1% retinoic acid in equal parts of 95% ethanol and propylene glycol. After only 4 weeks of local therapy a dramatic cosmetic result was evident, with marked resolution and flattening of the involved area. Subsequent application every 2 to 3 days has prevented recurrence.

Patient 2.—A male aged 14 presented with grouped open comedones on the right chest, extending in a line down the medial aspect of the right arm. No other developmental abnormalities were present. Treatment consisted of daily application of 0·1% retinoic acid in equal parts of 95% ethanol and propylene glycol. After 3 weeks, the impacted horny material had been extruded, with minimal irritation in the treated areas. Application of the retinoic acid every 3 days has prevented recurrence of the comedones.

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DISCUSSION

Naevus comedonicus results from a localized defect in the development of hair follicles (Beerman and Homan, 1959). Microscopically, deep epithelial invaginations are found surrounding a densely packed keratinous material. This invaginated epithelium appears to be differentiating towards follicular epithelium. This incompletely differentiated epithelium produces horny squamae which are stuck tightly together to form a dense plug in which the individual cells cannot dehisce. As the plug increases in size, the follicle is distended and the clinical picture of a comedo emerges.

Retinoic acid has recently been shown to be an extremely effective agent in ridding the follicles of horny impactions from acne vulgaris (Kligman *et al.*, 1969) and keratosis follicularis (Fulton *et al.*, 1968). The similarity of the clinical and histological findings in naevus comedonicus and the open comedones of acne vulgaris prompted a trial of retinoic acid in our patients. Both showed dramatic improvement in 3 to 4 weeks and were virtually clear by 5 weeks. Neither patient reported any discomfort from using this agent. Periodic continued application of the retinoic acid has prevented any reformation of the comedonal impactions.

Retinoic acid is an irritant to the skin, and its effect on comedones appears to be related to irritation of the follicular epithelium. The follicular lining of comedones treated with retinoic acid shows increased proliferative activity, manifested by acanthosis, parakeratosis and a great increase in the uptake of tritiated thymidine (Kligman *et al.*, 1969). The result of this increased follicular turnover is the production of a loose, less coherent horny material which is then able to be desquamated through the follicular orifice. Continued use of retinoic acid is necessary to prevent the reformation of a horny plug. Because it is an irritant, many patients develop significant erythema and may even go through an early period of pustule formation during the treatment of acne. Neither patient in this study experienced any irritation.

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COSMETIC CAMOUFLAGE IN DERMATOLOGY

T. W. STEWART AND DOREEN SAVAGE

Summary.—This paper advocates and illustrates the use of a camouflage clinic for patients with embarrassing skin blemishes, especially where severe psychological stress has resulted. The staff of such a clinic should have special training in the techniques employed.

FROM far in antiquity, man has coloured his skin with a variety of paints and dyes in order to enhance his beauty, or frighten his enemies.

In this century the adornment aspect has called forth the thriving cosmetics industry, which uses all our modern technological resources to produce innumerable preparations in which varied and interesting claims are made: to remove crow's feet, rejuvenate the skin, slow down the rate of appearance of ageing phenomena, and so on. Many of these cosmetics, however, do no more than cover up the alleged flaw in the integument, and it was the realization of this fact which led to work to be described in this paper. Why not take the principle further, and try deliberately to conceal such major blemishes of the skin, as interfere with a person's life and, maybe, livelihood?

Many special cosmetics are now available for making-up the face, and these can be used by a skilled beauty therapist, specially trained in cosmetic camouflage, to make a new face. Faces can be changed (e.g. for those with unsightly naevi, vitiligo or other pigmentary changes), restored (for those whose face has been damaged by accident, burning or surgical scarring), and improved (for those with slight damage, e.g. from acne scarring or skin grafts). In these days of the bikini and the mini-skirt, it is not only the face which attracts attention; blemishes on other parts of the body can cause distress and require treatment, e.g. telangiectasia or naevi on the legs and trunk, and erythema ab igne.

Basically, the cosmetic camouflage expert is a beauty therapist who has had special training and experience in the art of masking skin blemishes of all kinds. He or she needs to have a sound knowledge of the skin, and wide information about cosmetics currently available, their ingredients and price. The latter is most important because the creams are not available for prescription through the National Health Service (except under exceptional circumstances).

Special training is required because techniques additional to those learned in the standard training of the beauty therapist are necessary so that, after the defects have been obscured by the skilful use of a minimum of ordinary make-up, skin texture and colour are balanced to the point where no inequality whatsoever is apparent. The judicious use of make-up can also create, from some other, perhaps previously ignored, good feature, a new focus of attention. In patients with chronic discoid lupus erythematosus, which causes skin pitting and scarring,

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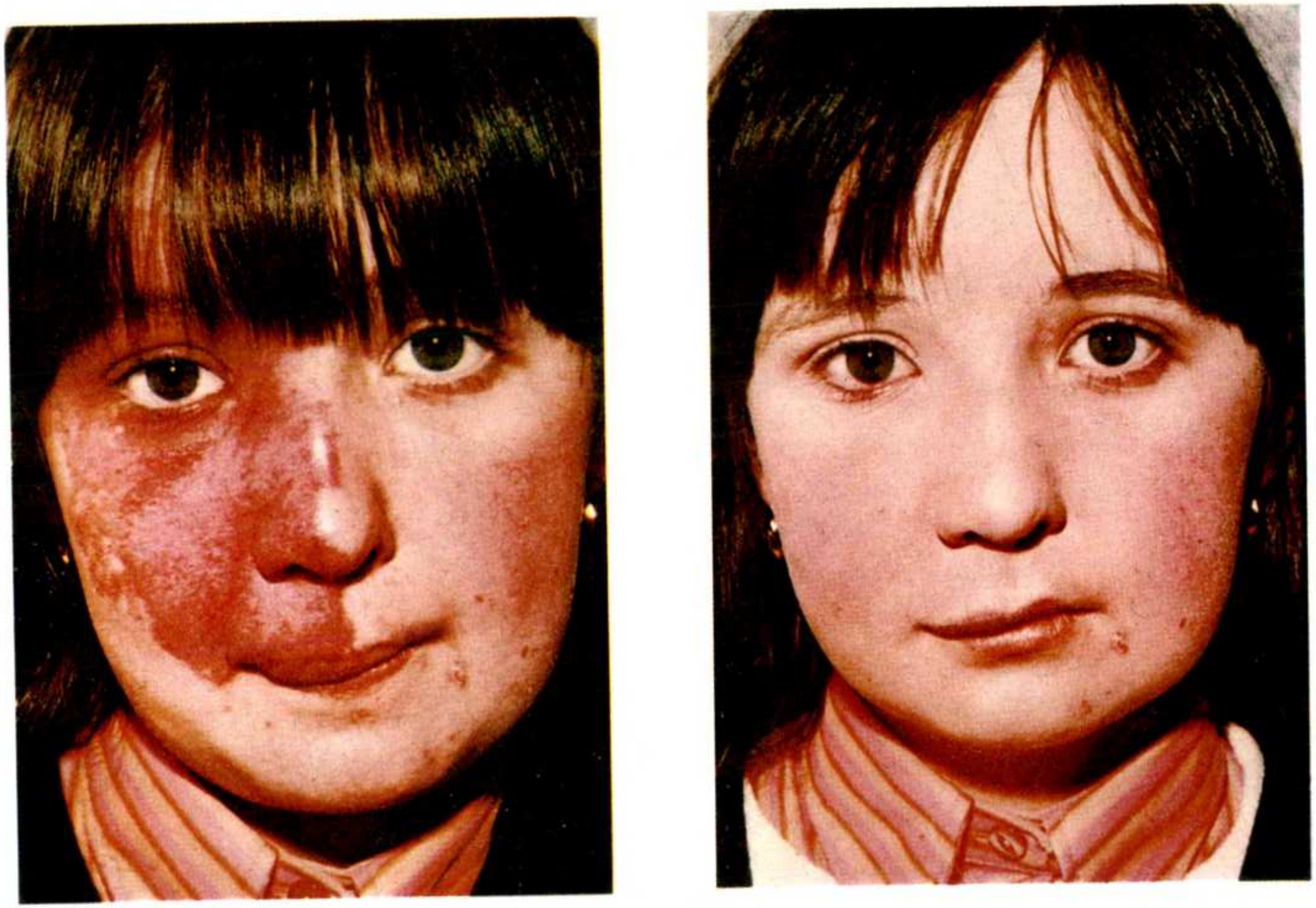

FIG. 1, 1a.—Port wine stain, before and after camouflage application (child).

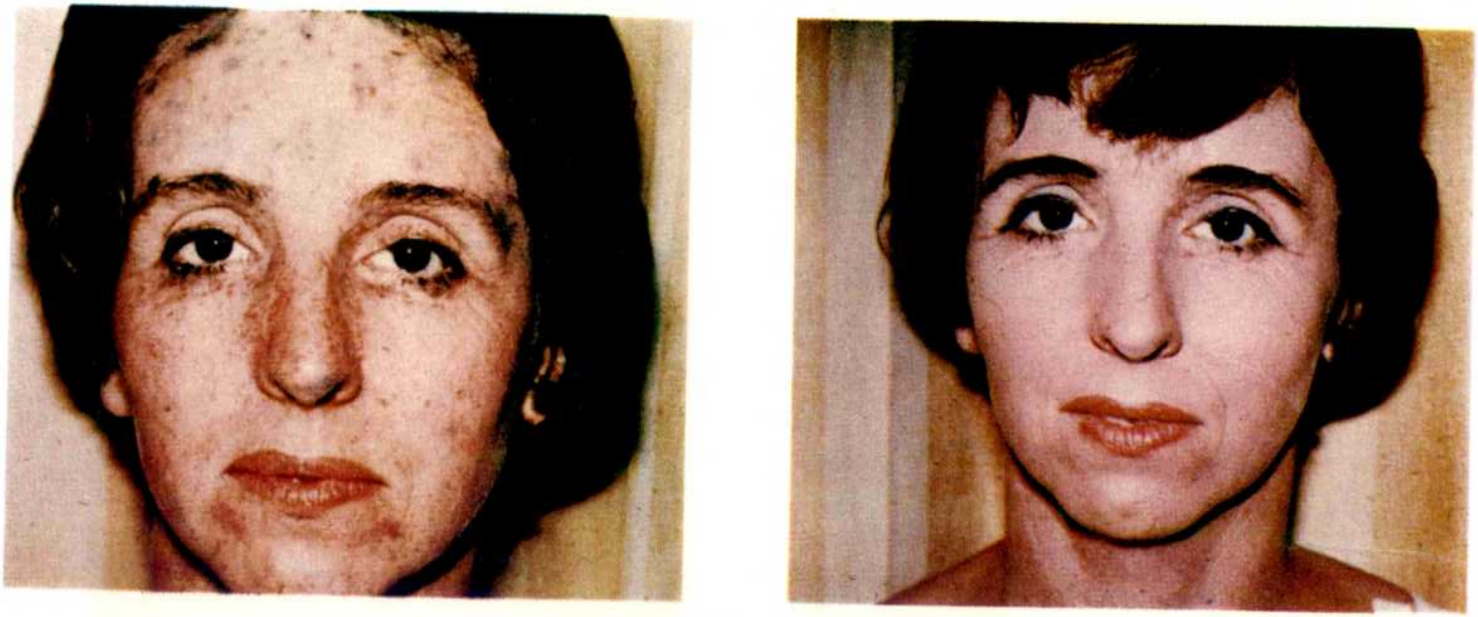

FIG. 2, 2a.—Lupus pernio of Hutchinson, before and after camouflage application (adult).

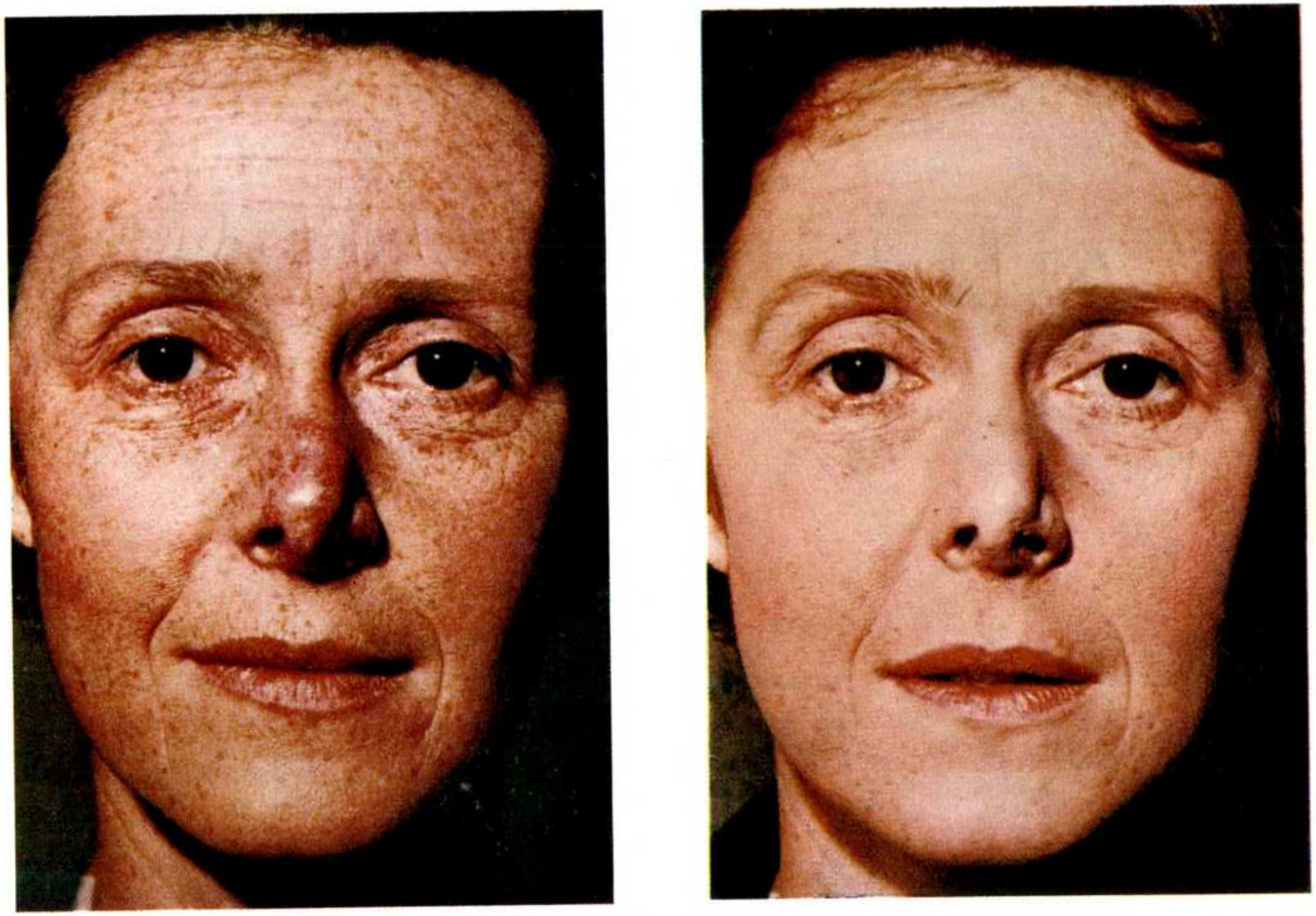

Fig. 3, 3a.—Multiple naevi, before and after camouflage application (adult).

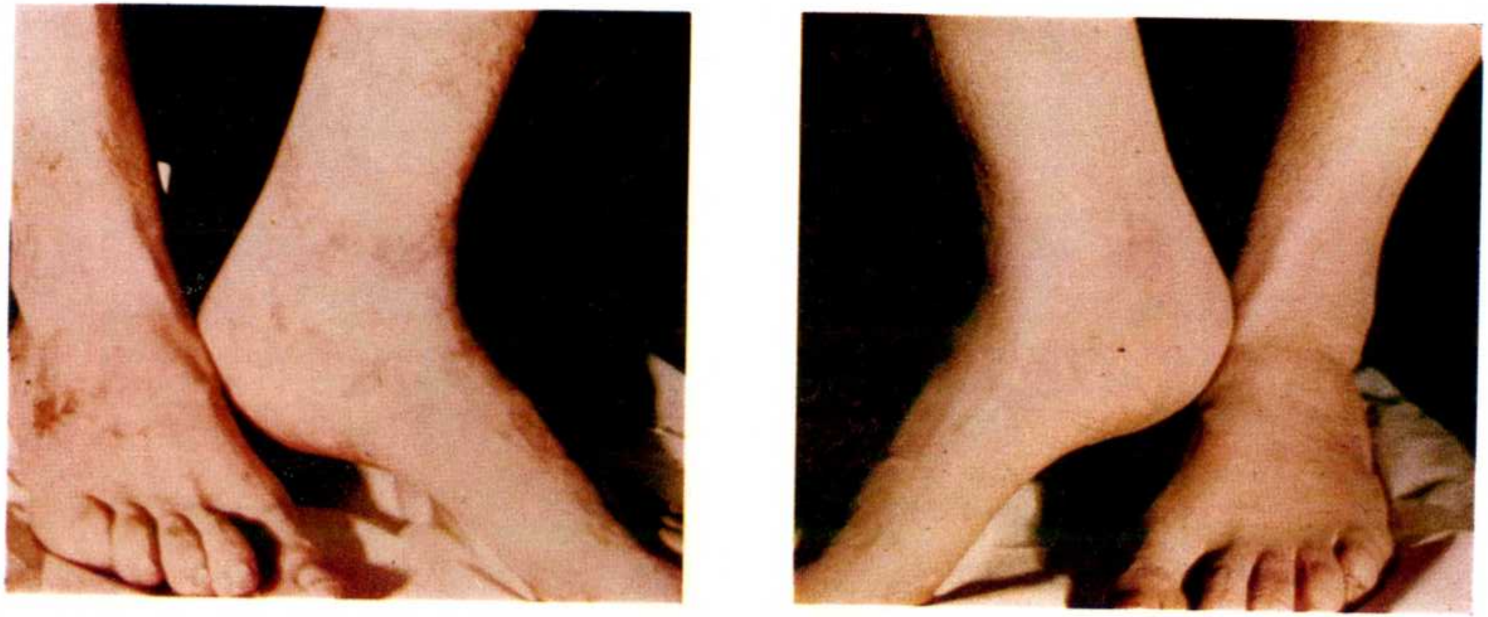

FIG. 4, 4A.—Telangiectatic naevus of leg. before and after camouflage application (adult).

build-up techniques, followed by stippling, can be carried out. Artificial freckles may need to be added to restore balance, and even " five-o-clock shadow " can be produced.

The camouflage technique depends basically on the use of a number of opaque covering creams which are non-irritant and have noticeably better finish and durability when compared with the standard cosmetics. These are blended on the skin to achieve a perfect match with the surrounding area, and then set, with a special untinted, unperfumed finishing powder. The results can be extremely natural. The delicate texture of a child's complexion can be closely matched; darker skin tones present no problem, and even men can conceal facial blemishes without fear of appearing to be " made-up ". One application lasts all day, and some of the creams are waterproof, so that the wearer really can face the rigours of our uncertain climate with impunity. Many of the results can be considered excellent, with complete and natural masking of the lesion. Generally speaking, the more irregular or pitted the skin surface, the more difficult it is to achieve a satisfactory finish. However, once the correct blend of preparations has been achieved, the patient rapidly acquires skill in applying his or her camouflage, so that even fairly complex routines need take no more than 15 minutes or so each morning. A successful change of face can sometimes bring increased self-confidence and other psychological advantages.

The following case histories are included to illustrate the type of patient treated:

Case 1.—*L.M.*

This youth aged 21 has an extensive port-wine stain, involving most of the right side of his face and neck, which caused him so much embarrassment that he felt he could not face people. He walked around with a stooping posture, tucking the affected side of his face into his chest. He also suffered from severe psoriasis. When he emerged from his first period of camouflage instruction, his alteration in bearing was dramatic. He stood upright, had a more confident aspect, and was obviously overjoyed. This change in outlook persisted, so much so, that when shown to a clinical meeting, his general practitioner, who was present and knew him well, failed to recognize him. His psoriasis has cleared completely and he now has a job, which previously he had not.

Case 2.—*E.T.*

This man aged 58 had suffered from chronic discoid lupus erythematosus of the face and scalp, which was inactive, but which had left extensive and disfiguring scarring. The embarrassment caused by his appearance was so great that he had retired and led the life of a recluse. At the end of the course of training in the technique of camouflage, he was so delighted with his new facial aspect that he returned to an active working life. Because of the extensive damage to the skin surface, the end result is by no means as good as could be obtained in other conditions, but, nevertheless, is very satisfactory.

Case 3.—*Miss G.K.*

This patient aged 19 attended the clinic because of pruritus ani. During the history-taking, it emerged that she had been a very active swimmer, but, because of the development of a telangiectatic naevus along almost the whole length of her left leg, she had given up her favourite sport, and felt the loss keenly. Following the use of a waterproof covering cream, which obliterated the lesions entirely, she is again able to wear a swim-suit with confidence, and has taken up swimming once more. Her pruritus ani has also disappeared.

Case 4.—*Mrs H.F.*

This lady aged 56 is the wife of a senior university academic. As such, she had a very active social life, which became curtailed, following the development of lupus pernio of Hutchinson, with a particularly deep erythrocyanotic tint. Topical and intralesional steroids had no effect, and the lady became very depressed. However, the successful use of camouflage technique has revolutionized her social life, in as much as she is now confident enough to take up the threads of it where she left off.

Case 5.—*Miss K.McC.*

This patient aged 43 developed lupus vulgaris at the age of 3½, which, despite lengthy treatment, had left her with extensive facial scarring. Her personality gradually deteriorated, until she had to seek psychiatric aid, which helped.

Eventually she was referred to the Camouflage Clinic, and the combination of encouragement, general advice about dress and make-up, and, especially, her very skilful adoption of a camouflage technique, have resulted in a striking improvement in her appearance and her morale. She can now make herself look most attractive, and at last has been able to hold down a job for longer than a month.

The writers would like to acknowledge the help received from the following in the form of clinical details and photographs: Dr K. D. Crow, Consultant Dermatologist, Princess Margaret Hospital, Swindon; Professor J. A. Milne, Department of Dermatology, University of Glasgow; Dr C. F. H. Vickers, Department of Dermatology, University of Liverpool.

History of Dermatology

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SHAKESPEARE ON THE SKIN

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THERE is an extensive literature on the medical aspects of Shakespeare's works (e.g., Bucknill, 1860; Simpson, 1959). Indeed, Edgar (1970) lists over 600 references in his very comprehensive review of this subject. Several writers have linked Shakespeare's medical observations with various medical disciplines, such as pharmacology (Macht, 1918), paediatrics (Goldbloom, 1936; Simpson, 1959), chest diseases (Vest, 1950), rheumatology (Ehrlich, 1967) and obstetrics (Kaltreider, 1964).

The many references in the works of Shakespeare to the skin, both normal and abnormal, have received little attention from commentators. The only analysis, albeit oblique, was by Davis (1969) when she demonstrated that many of Shakespeare's dermatological writings were in fact " cutaneous curses ".

In this paper I will attempt to interpret some of Shakespeare's comments on the skin in modern dermatological terms. Such interpretation is fraught with difficulties. Thus, although Shakespeare makes many references to leprosy, this disease was not accurately distinguished from certain other diseases until 1847 (Browne, 1970), and terminological confusion prevailed until quite late in the nineteenth century. Similar difficulties arise with other terms used by Shakespeare, such as scurvy, measles, serpigo and tetter (*vide infra*).

I shall comment on some of Shakespeare's writings on normal and abnormal skin, wounds and therapy. This short review is in no way comprehensive.

ON NORMAL SKIN

There's no art
To find the mind's construction in the face:
Macbeth, I, iv

Shakespeare makes numerous references to the face. The appearances of youth:

And then the whining school-boy, with his satchel,
And shining morning face, creeping like snail
Unwilling to school.
As You Like It, II, vii

are contrasted with that of old age:

Have you not a moist eye, a dry hand, a yellow cheek,
a white beard, a decreasing leg, an increasing belly?
2 King Henry IV, II, ii

Shakespeare here is surely describing, poetically, senile elastosis with possible ectropion.

Several stimuli capable of producing pallor of the skin are clearly described:

> I shall see thee, ere I die, look pale with love.
> With anger, with sickness, or with hunger, my lord,
> not with love:
> *Much Ado About Nothing*, I, i

The effect of fear on the skin and other organs receives attention:

> Cold fearful drops stand on my trembling flesh.
> What! do I fear myself? there's none else by:
> *King Richard III*, V, iii

and

> I am surprised with an uncouth fear;
> A chilling sweat o'er-runs my trembling joints:
> My heart suspects more than mine eye can see.
> *Titus Andronicus*, II, iii

Virility, facial hair and cowardice are neatly linked:

> How many cowards, whose hearts are all as false
> As stairs of sand, wear yet upon their chins
> The beards of Hercules and frowning Mars,
> Who, inward search'd, have livers white as milk;
> *The Merchant of Venice*, III, ii

The bard gives a poetic prognosis to greying of the hair:

> And these grey locks, the pursuivants of death,
> *1 King Henry VI*, II, v

Shakespeare is most accurate in his prognosis of male pattern alopecia:

> There's no time for a man to recover
> his hair that grows bald by nature.
> *The Comedy of Errors*, II, ii

However, he does soften the blow for those affected, perhaps because he was himself balding, judging from his portrait and bust:

> Why is Time such a niggard of hair being, as it is,
> so plentiful an excrement?
> Because it is a blessing that he bestows on beasts:
> and what he hath scanted men in hair he hath given
> them in wit.
> Why, but there's many a man hath more hair than wit.
> Not a man of those but he hath the wit to lose his hair.
> Why, thou didst conclude hairy men plain dealers
> without wit.
> *The Comedy of Errors*, II, ii

Finally, Shakespeare describes colourfully the effect of dieting:

> Bardolph, am I not fallen away vilely since this last
> action? do I not bate? do I not dwindle? Why, my
> skin hangs about me like an old lady's loose gown:
> I am withered like an old apple-john.
> *1 King Henry IV*, III, iii

CLINICAL DESCRIPTIONS, OBSERVATIONS AND APHORISMS

> Diseased nature oftentimes breaks forth
> In strange eruptions;
> *1 King Henry IV*, III, i

Shakespeare's clinical descriptions and aphorisms reflect mainly the killing and maiming diseases, which he must not only have seen, but also read about and discussed during his lifetime. Recurring themes are plague and pestilence, sweating sickness and fever, scurvy, measles, leprosy and the pox. Gout, rheumatism and wounds also receive comment.

The number of medical references in Shakespeare's works has suggested to some commentators that Shakespeare must have been a medical practitioner (e.g. Kaltreider, 1964), but there is no evidence for this supposition (Simpson, 1959; Edgar, 1970). Furthermore, there is no evidence that John Hall, Shakespeare's son-in-law and an eminent local practitioner of the day, influenced the medical content of Shakespeare's works (Simpson, 1959).

Medical knowledge was diffused among laymen to a much greater degree in Shakespearian times than it is today, because it was less " technical " and based largely on literary sources (Edgar, 1970). The medical references, which are not only numerous in Shakespeare's plays, but also in the plays of his contemporaries, are a reflection of this fact.

The following passage illustrates both the range of diagnoses current at the time and how effectively Shakespeare used these diagnostic terms as a curse.

> Now the rotten diseases of the south, the guts-
> griping, ruptures, catarrhs, loads o'gravel
> i' the back, lethargies, cold palsies, raw eyes,
> dirt-rotten livers, wheezing lungs, bladders full
> of imposthume, sciaticas, lime-kilns i' the palm,
> incurable bone-ache, and the rivalled fee-simple
> of the tetter, take and take again such preposterous
> discoveries!
> *Troilus and Cressida*, V, i

Lime-kilns i' the palm may be a poetic reference to pompholyx, whilst tetter, in Shakespeare's day, referred to skin diseases in general, ranging from ringworm, eczema and herpes to pustules, ulcers and blisters (Davis, 1969).

In the following lines, Simpson (1959) regards the following reference to tetter as indicating an acute exfoliative dermatitis:

> And a most instant tetter bark'd about,
> Most lazar-like, with vile and loathsome crust,
> All my smooth body.
> *Hamlet*, I, v

The word lazer was applied to a leper. Singer and Underwood (1962) assert that " The medieval treatment of lepers was one of the dark incidents of man's inhumanity to man ". The attitude at the time is expressed by Shakespeare on several occasions. Thus:

> Ask me not what I would be, if I were not Thersites,
> for I care not to be the louse of a lazar.
> *Troilus and Cressida*, V, i

and the fact that leprosy was held in contempt, by:

> A plague on thee! Thou art too bad to curse.
> All villains that do stand by thee are pure.
> There is no leprosy but what thou speak'st.
> *Timon of Athens*, V, iii

It was not only those with leprosy, or skin disease resembling leprosy, who were likely to suffer in Shakespeare's day. People with unusual birthmarks were likely to be suspected of being witches (Rowse, 1971). The silent thoughts of a Shakespearian bride become more poignant when this fact is taken into account.

> And the blots of Nature's hand
> Shall not in their issue stand:
> Never mole, hare-lip, nor scar,
> Nor mark prodigious, such as are
> Despised in nativity,
> Shall upon their children be.
> *A Midsummer-Night's Dream*, IV, i

The ravages of syphilis were well known to Shakespeare, who accurately describes tertiary syphilis and its accompanying alopecia, thus:

> In hollow bones of man; strike their sharp shins,
> And mar men's spurring. Crack the lawyer's voice,
> That he may never more false title plead,
> Nor sound his quillets shrilly: hoar the flamen,
> That scolds against the quality of the flesh,
> And not believes himself: down with the nose,
> Down with it flat; take the bridge quite away
> Of him that, his particular to foresee,
> Smells from the general weal: make curl'd-pate
> ruffians bald;
> And let the unscarr'd braggarts of the war
> Derive some pain from you: plague all,
> That your activity may defeat and quell
> The source of all erection. There's more gold;
> Do you damn others, and let this damn you,
> And ditches grave you all!
> *Timon of Athens*, IV, iii

A wheal (or weal), in Shakespeare's day was probably used to describe a pustule (*Lancet*, 1971), and hoar means " afflict with white leprosy " (Davis, 1969).

The alopecia of secondary syphilis was sometimes referred to by Shakespeare as a " French crown ". In the following lines this description is directed against the French troops by King Henry V, in a particularly witty fashion:

> Indeed, the French may lay twenty French crowns to one,
> they will beat us; for they bear them on their shoulders:
> but it is no English treason to cut French crowns, and
> tomorrow the king himself will be a clipper.
> *King Henry V*, IV, i

Plague was an ever present threat, and Shakespeare's writings indicate that he knew that the presence of purpura indicated a fatal outcome. This purpuric eruption was referred to by Shakespeare as " death's tokens ", " Lord's tokens " or " red pestilence ". The following lines illustrate this point:

> He is so plaguy proud that the death-tokens of it
> Cry " no recovery "
> *Troilus and Cressida*, II, iii

and:

> Now the red pestilence strikes all trades in Rome,
> And occupations perish!
> *Coriolanus*, IV, i

In relating Falstaff's death, the Hippocratic facies are described in 4 lines by Shakespeare, with his great genius for language:

> for after I saw him fumble with the sheets and play
> with flowers and smile upon his fingers' ends,
> I knew there was but one way; for his nose was
> as sharp as a pen, and a'babbled of green fields.
> *King Henry V*, II, iii

This description so closely parallels in a factual sense the description by Dr Peter Lowe in his English translation of Hippocrates' *Prognosticon*, published in London in 1597 (McNair, 1969) that Shakespeare may have been using this work as his reference. Indeed, it is very likely that Shakespeare was familiar with medical books and the doctors of the period (Edgar, 1970; Richardson, 1971). Shakespeare intended Falstaff to die of the " sweat ". Sweating sickness was eloquently described by Dr Caius in 1552. The nature of this disease is unknown; the last recorded epidemic occurred in 1551 (Roueché, 1963).

The word scurvy was used by Shakespeare mainly to curse. Thus the nurse remarks:

> Scurvy knave! I am none of his flirt-gills:

and

> Now, afore God, I am so vexed, that every part
> above me quivers. Scurvy knave!
> *Romeo and Juliet*, II, iv

The term was used loosely by Shakespeare, and it was not until 1593 that a good clinical description of scurvy was given by Sir Richard Hawkins, followed by John Woodall in 1617. However, later physicians were confused about the clinical features of this disease, until Lind redressed the balance in 1753 (Keynes, 1967). It is of interest that Shakespeare's son-in-law, Dr John Hall, was thought a little unusual by his fellow physicians in prescribing scorbutics, but he clearly describes scurvy in his wife (Simpson, 1959).

Measles was used in Shakespeare's time for leprosy or veneral disease (Davis, 1969). The following lines, probably referring to veneral disease, summarize the difficulties in interpretation:

> Coin words till their decay against those measles,
> Which we disdain should tetter us, yet sought
> The very way to catch them.
> *Coriolanus*, III, i

Although the Arabic writer Rhazes (850–932) made a clear distinction between smallpox and measles, this work was later neglected (Copeman and Copeman, 1970a) and separation of measles proper from other fevers was not made until about 1670 by Thomas Sydenham (Lynch, 1969). Shakespeare's use of the word clearly reflects this fact.

It is a tribute both to Shakespeare's powers of observation and to his great industry that, whilst living in a society constantly threatened by killing diseases, he found time to describe some minor dermatological disorders. Thus scabies is recorded:

> a small grey-coated gnat,
> Not half so big as a round little worm
> Prick'd from the lazy finger of a maid;
> *Romeo and Juliet*, I, iv

and even corns:

> Welcome, gentlemen! ladies that have their toes
> Unplagued with corns will have a bout with you.
> Ah ha! my mistress, which of you all
> Will now deny to dance? she that makes dainty, she
> I'll swear, hath corns; am I come near ye now?
> *Romeo and Juliet*, I, v

Herpes labialis is described with a suggestion of possible aetiological factors:

> O'er ladies' lips, who straight on kisses dreams;
> Which oft the angry Mab with blisters plagues,
> Because their breaths with sweetmeats tainted are.
> *Romeo and Juliet*, I, iv

The clinical features of rosacea are beautifully expressed:

> one Bardolph, if your majesty know the man:
> his face is all bubukles, and whelks, and knobs,
> and flames of fire; and his lips blows at his nose,
> and it is like a coal o'fire; sometimes plue and
> sometimes red;
> *King Henry V*, III, vi

" Bubukle " is a combination of carbuncle and bubo, and is used to describe a pimple. " Knobs " refer to any protuberance and in particular to small tumours, cysts or pimples. " Whelks " are pustules or pimples (Davis, 1969). " Plue " means blue. I would like to think that " lips blows at his nose ", may indicate an associated rhinophyma.

Shakespeare also hazards a possible aetiological factor:

> O! thou art a perpetual triumph, an everlasting
> bonfire light. Thou hast saved me a thousand marks
> in links and torches, walking with thee in the night
> betwixt tavern and tavern: but the sack that thou hast
> drunk me would have bought me lights as good cheap
> at the dearest chandler's in Europe.
> *1 King Henry IV*, III, iii

Acne vulgaris receives little attention, and it is interesting that youth in Shakespeare's plays is usually portrayed " rose-faced ", as for example when the ravages of syphilis:

> bring down the rose-cheeked youth
> To the tub-fast and the diet.
> *Timon of Athens*, IV, iii

However, the following lines are thought to refer to acne vulgaris (Davis, 1969):

> O, sir! upon her nose, all o'er embellished
> with rubies, carbuncles, sapphires, declining
> their rich aspect to the hot breath of Spain,
> who sent whole armadoes of caracks to be ballast
> on her nose.

Precious stones were sometimes referred to as carbuncles. Did Shakespeare recognize the seborrhoea characteristic of acne? The following line dealing with this same unfortunate young woman would suggest he did:

> Marry, sir, she's the kitchen-wench, and all grease,

Some of her greasiness may be attributed to her occupation as a kitchen maid and perhaps to her obesity, thus:

> and I know not what use to put her to but
> to make a lamp of her and run from her by
> her own light. I warrant her rags and the
> tallow in them will burn a Poland winter:

and later her obesity is stressed:

> she is spherical, like a globe:

The idea that some of her acne, if we accept the diagnosis, is due to dirt is expressed:

> Swart, like my shoe, but her face nothing like so clean kept:

Swart may be interpreted as grimy. The idea that this grime would not disappear after washing with water is also expressed:

> That's a fault that water will mend.
> No, sir, 't is in the grain; Noah's flood could not do it.
>
> All these quotations—*Comedy of Errors*, III, ii

Serpigo was a term used by Shakespeare to denote either shingles or psoriasis (Davis, 1969). The following line may refer to psoriasis, as the serpigo is described as dry:

> Now, the dry serpigo on the subject!
> *Troilus and Cressida*, II, iii

Serpigo is also used by Shakespeare in the following lines:

> Friend hast thou none;
> For thine own bowels, which do call thee sire,
> The mere effusion of thy proper loins,
> Do curse the gout, serpigo, and the rheum,
> For ending thee no sooner.
> *Measure for Measure*, III, ii

Here Shakespeare has linked gout, rheumatism and possibly psoriasis. Could this be the first recognition of psoriatic arthropathy? This may be the case, but there are 2 minor reservations which should be considered before reaching such a conclusion.

First, the serpigo mentioned is not necessarily of the dry variety and may, therefore, be interpreted as shingles. It does seem odd, however, to place shingles between gout and rheumatism, unless the common denominator intended by Shakespeare was pain.

Secondly, the term rheum was used by Shakespeare not only to denote joint swelling, but also with reference to a watery discharge due to lacrimation, or salivation, or to the common cold (Ehrlich, 1967). Thus rheum in this instance could qualify serpigo to denote a weeping herpes zoster, but this seems unlikely.

I would like to think that Shakespeare may have recognized psoriatic arthropathy. However, it may be that the poet merely chose at random 3 common, chronic and untreatable conditions to make his point.

ON WOUNDS

Shakespeare makes many comments on wounds; these include:

> He jests at scars that never felt a wound.
> *Romeo and Juliet*, II, i

and

> How poor are they that have not patience!
> What wound did ever heal but by degrees?
> *Othello*, III, ii

I particularly like:

> O! then beware;
> Those wounds heal ill that men do give themselves:
> *Troilus and Cressida*, III, iii

Wounds becoming green, and therefore infected, were thought to carry a favourable prognosis. Thus:

> Send succours, lords, and stop the rage betime,
> Before the wound do grow incurable;
> For, being green, there is great hope of help.
> *2 King Henry VI*, III, ii

and

> Did not goodwife Keech, the butcher's wife,
> come in then and call me gossip Quickly?
> coming in to borrow a mess of vinegar; telling
> us she had a good dish of prawns; whereby thou
> didst desire to eat some, whereby I told thee
> they were ill for a green wound?
> *2 King Henry IV*, II, i

ON THERAPY

What of therapy in Shakespeare's plays? Most therapy during the poet's lifetime revolves around diet and evacuation, although some external applications were used (Copeman and Copeman, 1970b). Superstition was central to many of the ideas about disease and its treatment. Shakespeare illustrates this well by his description of " touching for the evil ".

Macduff and Malcolm fled from the tyranny of Macbeth to the English court of Edward the Confessor. The monarch was believed to possess the power to cure the " King's Evil ", or scrofula, simply by touching those affected:

Macduff: What's the disease he means?
Malcolm: 'Tis called the evil:
A most miraculous work in this good king,
Which often, since my here-remain in England,
I have seen him do. How he solicits heaven,
Himself best knows; but strangely-visited people,
All swoln and ulcerous, pitiful to the eye,
The mere despair of surgery, he cures,
Hanging a golden stamp about their necks,
Put on with holy prayers; and 't is spoken
To the succeeding royalty he leaves
The healing benediction.
Macbeth, IV, iii

It may be that the hanging of a golden stamp around the neck was as much sought after as the monarch's touch, and perhaps as effective in curing the " evil ".

Some idea of what was used to dress the skin can be gained from Shakespeare's writings. Thus for cuts:

I shall desire you of more acquaintance,
good Master Cobweb: if I cut my finger,
I shall make bold with you.
A Midsummer-Night's Dream, III, i

and for a bleeding face:

Go thou; I'll fetch some flax and white
of eggs
To apply to his bleeding face. Now,
heaven help him!
King Lear, III, vii

and

Your plantain leaf is excellent for that.
For what, I pray thee?
For your broken shin.
Romeo and Juliet, I, ii

Shakespeare also refers to the use of parmaceti:

And telling me the sovereign'st thing on earth
Was parmaceti for an inward bruise;
1 King Henry IV, I, iii

Parmaceti was advocated for the treatment of spots and morphew (ringworm) by John Woodall, a seventeenth-century surgeon (Simpson, 1959).

The treatment of syphilis was horrific:

Be a whore still; they love thee not that use thee;
Give them diseases, leaving with thee their lust.
Make use of thy salt hours; season the slaves
For tubs and baths; bring down rose-cheeked youth
To the tub-fast and the diet.
Timon of Athens, IV, iii

The patient was placed in a tub and exposed to the fumes created by powder of cinnebar, thrown on to a hot plate. This was described as a " deadly medicine " by Woodall (Simpson, 1959).

CONCLUSION

I have attempted to interpret Shakespeare's writings on the skin in terms of modern dermatology. Although there are considerable difficulties in reaching valid conclusions, Shakespeare describes a surprising variety of dermatological conditions with great clarity. Elizabethan ideas about both skin disease and normal skin are reflected in the poet's works, and shed some light on the practice of medicine during his lifetime.

A Coxsackie B2 virus made this study possible.

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My wife kindly typed the manuscript.

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Comment

THE ROLE OF CHALONES IN EPIDERMAL HOMEOSTASIS

Keratinocytes are not long-lived cells. They move upwards from their point of generation in the basal layer to the top of the granular cell layer in 14 days and thence to eventual desquamation at the top of the stratum corneum in a further 14 days. It should be said that this " turnover " of 28 days has recently been questioned by Halprin (1972) who believes that wrong conclusions have been drawn from previous autoradiographic data and that the real normal turnover time is 40–56 days. Whether the correct figure is 28 or 56 days, basal cells must steadily divide to provide keratinocytes to replace those shed at the surface as horny squames. Despite inevitable variations in the rate of desquamation from daily minor traumata, changes in humidity, and changes in the mitotic rate resulting from fluctuating levels of circulating hormones the thickness of the epidermis remains constant. In addition, after injury there is a rapid architectural rearrangement of the epidermis with accompanying bursts of mitotic activity in the process of wound healing. The constancy of the structure of the epidermis and its ability rapidly to reconstitute itself after injury imply the existence of a control mechanism for division of epidermal cells. The possible relevance of this control mechanism to disorders of epithelial proliferation, including psoriasis and neoplasia has led to intensive study of epidermal cell kinetics in the past two decades. Amongst control systems proposed, especial attention should be given to those involving the negative feedback principle as this is a biologically popular way of providing a delicate servo-mechanism. The most widely known examples of such a negative feed back system are probably those governing the inter-relationships of the pituitary with the adrenals, thyroid and gonads. Bullough and Laurence (1960a) published the results of ingenious experiments in which small breaches of epidermis were produced on one side of the ears of mice. By observing the rise in the mitotic rates at the edges of the wound and in the epidermis on the other side of the very thin mouse ear they felt that they were able to dismiss the idea of a stimulatory wound hormone and they invoked the concept of a locally produced tissue hormone that exerted an inhibitory influence on mitosis in that tissue which they termed " chalone ". It was suggested that this inhibitory substance was normally produced in the differentiated epidermis and when the differentiated keratinocytes were injured or lost by wounding the inhibitor that was normally produced was lost, allowing a burst of mitotic activity in the adjacent tissue. This " chalone " hypothesis was in accord with a general growth control mechanism proposed earlier by Weiss and Kavenau (1957). These authors suggested that each differentiated cell in a growing tissue produced specific freely diffusible compounds (anti-templates) antagonistic to specific key compounds (templates) that catalysed the " generative mass ".

Bullough and Laurence (1961a) described their modification of a simple *in vitro* system for detecting and investigating the inhibitory activity of extracts of epidermis. In this model the mitotic rates in mouse ear epidermis were calculated from counting mitoses arrested in metaphase by the stathmokinetic agent colcemid. " Chalone " activity was regularly found in the aqueous supernatant of homogenates of the skin of the adult mouse by these workers using the experimental model that they had described. In a series of publications (Bullough and Laurence, 1960b, 1964a, b and 1966; Bullough *et al.*, 1964, 1967) the properties of the " epidermal chalone " were painstakingly plotted. They concluded that chalone activity resided in a protein or a glycoprotein material that was produced in and diffused from the differentiated portion of the epidermis and which was heat labile and tissue specific, but was not species specific. In addition, it appeared that the inhibitor substance required adrenalin and hydrocortisone for its full expression. Adrenalin was believed to be of especial importance in the production of a loose " chalone-adrenalin " complex and it was thought that the observed diurnal variation of epidermal mitotic rate could be explained by the daily fluctuations in the amount of circulating catecholamine (Bullough and Laurence, 1961a, 1964a). Not only was the inhibitory action evident in the *in vitro* system used, but it was also found *in vivo* in mice. Other tissues were also believed to have a similar servo-regulatory system for cell production, especially those that have of necessity a high rate of cell division. Organ specific mitotic inhibitors with the same general characteristics as the " epidermal chalone " have been described for the kidney (Simnett and Chopra, 1969), for the lung (Simnett *et al.*, 1969) and for the granulocyte series of white cells (Rytömaa and Kiviniemi, 1968a, b). In addition, it has been suggested that red cell production is also in part regulated by a "chalone" type of material (Seip, 1971). It is further suggested that " epidermal chalone " is strictly tissue specific and not organ specific, i.e. it has no action on the dendritic cell component of the epidermis. It certainly seems from recent work on the effect of melanoma extracts on other melanomata that there might be a separate specific melanocytic chalone substance (Bullough and Laurence, 1968b; Mohr *et al.*, 1968). More difficult to explain in terms of specificity is the supposed existence of a separate sebaceous gland chalone (Bullough and Laurence, 1970). The keratinocytes and sebaceous gland cells are both mature epidermal cells and chalone activity is specific indeed if separate inhibitory compounds are implicated in their reproductive economy. The existence of this sebaceous gland inhibitor was deduced from the disparity in depression of mitotic rates between epidermis and sebaceous glands when " pure epidermal chalone " was administered to mice as opposed to a similar amount of mitotic depression in epidermis and sebaceous glands when crude epidermal extracts were given.

The advantages of being able to investigate and use a pure preparation of epidermal chalone are evident. Initial attempts at separation showed that the mitotic inhibition induced by the supernatants of aqueous extracts of epidermis was destroyed by boiling and was recoverable in the precipitate formed by the addition of 70% alcohol (Bullough *et al.*, 1964). Later Boldingh and Laurence (1968) in the Organon Laboratories separated out an active inhibitory material from pig-skin powder using various fractionation methods. A suitable and effective method was found to be alcohol precipitation followed by column electrophoresis and then by dialysis. A similar material was obtained from cod skin in the same investigation. The mitotic inhibitory activity for epidermis was concentrated some 2000 times, but even so the authors admitted that the material they obtained might have been heterogenous. They believed it to be a protein or a glycoprotein with a molecular weight 30,000–40,000. A group of 4 articles evocatively entitled " Chalones and Cancer " appeared in *Nature* in 1968 and reported attempts at applying the concepts gained during investigations on normal tissue to some therapeutic use in the treatment of neoplastic disorders. The V×2 epidermal tumour of rabbits showed a marked reduction in the mitotic rate after treatment with a chalone preparation together with hydrocortisone and adrenalin (Bullough and Laurence, 1968a). Similar results were recorded for 2 types of melanoma using both *in vivo* and *in vitro* methods (Bullough and Laurence, 1968b; Mohr *et al.*, 1968), and for a granulocytic leukaemia (Rytömaa and Kiviniemi, 1968c). More recently other neoplasms have been reported as showing mitotic responsiveness to aqueous extracts of neoplastic tissue, the most recent being the Hewitt Keratinizing Epidermal Carcinoma of Mice (Bullough and Deol, 1971). In all these investigations the " chalone " extract appeared to produce mitotic inhibition both *in vivo* and *in vitro* and its activity depended on the simultaneous presence of adrenalin and hydrocortisone. In some instances not only was there mitotic responsiveness, but tumour destruction. It is interesting to speculate why extracts of the tumours themselves contained inhibitory activity. It was postulated that a common defect might be an inadequate intracellular concentration of the antimitotic chalone of the tissue from which the tumour originated. One theoretical fault in the chalone hypothesis as applied to neoplasms might be that chalones are supposed to emanate from mature differentiated tissue while cellularde differentiation is an accompaniment of most forms of neoplasia. There are also very real practical difficulties in translating the results of the experimental findings so far into useful treatments for human cancer as each neoplasm type would require an enormous amount of its own chalone preparation for any chance of eradicating the disease. For the foreseeable future, the " chalones " and cancer relationship is one of theoretical interest alone.

In order to utilize any " chalone effect " it is crucial to understand the point of action in the mitotic cycle and the mode of action of this class of compounds. This has proved to be one of the more difficult aspects of this type of research and conflicting results have been produced by different groups of workers. For example, Baden and Sviolka (1968) could not demonstrate a decrease in DNA synthesis in rat skin in short term experiments *in vitro*, using aqueous epidermal extracts and adrenalin. They concluded that the site of inhibitory action must be later in the mitotic cycle than DNA synthesis. However, Marks *et al.* (1971) demonstrated a decreased incorporation of tritiated thymidine in human skin *in vitro* after treatment with similar aqueous extracts for periods as short as 2 hours. Clearly this suggests that the inhibitory effect could result from an effect on the " S " phase, but as the culture systems and the species of the substrate tissue varied in these two investigations, it is difficult to compare them. A third group (Hennings *et al.*, 1969) injected mice with either crude aqueous extracts of skin or liver and then estimated the amount of tritiated thymine incorporated by the skin of the mice at differing intervals between injection and sacrifice. They found that although there was a rapid effect on mitosis, an effect on DNA synthesis was not apparent until 9 hours after treatment with the skin extract had elapsed. They suggested that an important aspect of the mode of action of "chalone " was on the point of entry of cells into the DNA synthesis phase.

In the past 2 years there have been some extremely exciting developments in the chalone story. Marrs and Voorhees (1971) described a modified bioassay technique for estimating mitotic inhibitory activity which attempted to standardize many of the variables that bedevil this type of study. They also used an improved preparation consisting solely of epidermis as the chalone rich material. A heat separation method was used for this purpose and was found to produce a split actually through the basal layer. As a " quantity control " check they also ascertained electron-microscopically that their homogenates showed complete destruction of cells and organelles. In other papers this same group (Powell *et al.*, 1971 and Duell *et al.*, 1971) report that beta-adrenergic substances (such as isoproterenol) produce stimulation of the enzyme adenyl cyclase within the epidermis and an increase in the amount of adenosine 3′5′ monophosphate (cyclic AMP) within the epidermis before mitotic inhibition. This is of great interest as the cyclic AMP adenyl cyclase system has recently been implicated as an intracellular pathway for hormonal stimulation of various types. Certainly, other physiological functions of adrenalin have been shown to be mediated by increased formation of cyclic AMP in other target tissues (Sutherland and Robison, 1966). Brønstad *et al.* (1971) have reported similar findings using adrenalin and hamster skin as the substrate tissue. These authors found that the lowest concentration necessary to stimulate adenyl cyclase in the epidermal preparation used was 10^{-7} mol/l which is higher than that found necessary to stimulate adenyl cyclase formation in other target tissues by their respective hormones. For this reason they suggested that the physiological activator of epidermal adenyl cyclase might be " another hormone of glandular or local origin ". On the basis of their own findings Duell *et al.* (1971) suggested that " the epidermal chalone system may be an epinephrine activated cascade of intra-epidermal molecular events ", in which the feedback control of epidermal cell division is mediated via cyclic AMP ". In another publication, Vorhees and Duell (1971) speculated that psoriasis may be a fault in the molecular system generating cyclic AMP within the epidermis. However, this aspect remains pure speculation as there is no firm evidence linking psoriasis to the adenyl cyclase-cyclic AMP system.

To summarize at this point, it may be said that there is considerable evidence for the existence of a chemically mediated negative feedback control mechanism for many mitotically active tissues including the epidermis However, whether the mitotic inhibition that results from the use of aqueous extracts of epidermis is due to a single chemical mediator such as " epidermal chalone " (albeit aided by adrenalin and hydrocortisone) or is a summation of the effect of several substances is less clear. Recently, Laurence and Randers-Hansen (1971) described different responses in tongue epithelium and ear epidermis to a chalone preparation, adrenalin and hydrocortosone in mice. Their study also investigated the effect of adrenalectomy on the results and from the lack of effect of this manoeuvre concluded that "the epidermal chalone may not act directly with adrenalin to cause a reduction in the mitotic rate ". Apart from this suggestion, the results led the authors to make the further proposition that there normally exists a chalone antagonist and that adrenalin inhibits this compound rather than assisting the chalone itself. It has also been suggested that the anti-chalone is produced in the dermis and that hydrocortisone suppresses the production of this chalone antagonist (Laurence *et al.*, 1972). That the regulatory system is probably more complex than first proposed has also been suspected by Elgjo *et al.* (1971). These authors reasoned that as, theoretically, chalone activity resides in the differentiated cells of the epidermis, if basal layer could be separated from the differentiated portion of the epidermis the maximal inhibitory activity should reside in the latter fraction rather than the former. By a technical *tour de force* they managed to accomplish this separation and found that surprisingly most of the inhibitory activity ended up in the basal layer fraction. These results led the authors to believe that " the process of cell division is regulated at different stages by different substances ". They suggest that there is a substance produced by basal cells that acts on cells in the G2 phase of the mitotic cycle and a second factor produced by the differentiated cells that regulates the rate of entrance of cells from G1 to the S phase It seems inevitable that more and more regulator materials will be described as more attention is paid to this field and the recent finding of a non-chalone type of inhibitor in tissue extracts of melanotic and amelanotic melanoma should not be greeted with surprise (Adachi *et al.*, 1971). This inhibitory material appears to be an RNA rather than a protein. An epidermal growth stimulating activity has also been described (Karasek and Charlton, 1971) which seems to emanate from dermal fibroblasts and it would not be surprising if other stimulatory factors were to be described in the near future. Leaving aside chemical influences, what other factors may influence epidermal cell renewal? One very important aspect to which little attention has been devoted is the role of cell contact in the control of mitotic rate. Carter (1968) has emphasized that cell contact, migration and cell division are closely interlinked phenomena for many tissues. It certainly seems possible that keratinocyte renewal could be geared in some way to corneocyte shedding and the loss of contact engendered by shedding could be the cornerstone in this sequence. Apart from " contact factors ", the blood supply, ambient temperature, circulating levels of such hormones as thyroxin and the sex hormones are amongst many other factors that must also influence epidermal turnover and cell replacement. It is very difficult at this time to be sure of the exact role of the chalone mechanism in the economy of the epidermis; it seems to be just one of many controlling influences and its exact place must await its chemical characterization.

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Books

Year Book of Dermatology. Edit. F. D. MALKINSON and R. W. PEARSON (1971). Chicago: Year Book Medical Publishers (London: Lloyd-Luke). Pp. 379, 59 illustrations. Price £6.80.

The 1971 Year Book appears in a new format, making its second change—the last was in 1950—towards a larger page and a slimmer volume. Malkinson and Pearson, who have replaced Kopf and Andrade as editors, have wisely made no important changes in the classification, which though somewhat empirical is now familiar to the many regular users of the year book. However, acne and acneiform eruptions now have a section of their own and the review articles and other additional references, formerly listed at the end, are now, for the greater convenience of the reader, attached to the sections of the book to which they refer.

The leading article is an excellent review of " Adverse Cutaneous Reactions to the Sun " by John H. Epstein. The selection of articles for inclusion is balanced and catholic and the comments by the editors or invited experts are almost all informative and stimulating. As usual the Year Book provides a valuable service for all English speaking dermatologists, and for the dermatologist who complains about the inadequacy of his libary facilities it is indispensable. Have the publishers considered the simultaneous publication of a Spanish edition?

ARTHUR ROOK

Dermatology in General Medicine. Edit. T. B. FITZPATRICK, K. A. ARNDT, W. H. CLARK, A. Z. EISEN, E. J. VAN SCOTT and J. H. VAUGHAN (1971). New York: McGraw-Hill. Pp. 2048, illustrated. Price £20.40.

When Radcliffe-Crocker first published his classic textbook of dermatology some 80 years ago he was able to cover the entire field comprehensively and effectively within the compass of 1400 pages and to cite a very large proportion of all the relevant literature. During the present century dermatology has advanced its frontiers to such an extent that all fields of medicine are overlapped to some degree. Moreover the full understanding of any dermatosis requires a knowledge in depth of parts of the territory of more than one of the basic sciences and often also of the technological changes which determine the patient's occupational and domestic environment. Dermatology in 1972 has reached the stage in its evolution at which it is confronted with the problems that general internal medicine first met 30 years ago and has still failed to solve. No one man can remain an authority on more than a part of the total field, keeping abreast of literature scattered through the journals of a dozen or more disciplines; for he must also maintain a reasonable level of diagnostic and therapeutic competence over the whole territory. It follows that no one man can write a comprehensive textbook at an advanced level unless it be exclusively clinical but must enlist the assistance of a team of specialists within the speciality. The organizing editor must thus decide for whom his book is intended, and must ensure that each contributor writes with the needs of these particular readers in mind. Dr Fitzpatrick, the senior of the 6 editors of this long awaited textbook, defines his objectives clearly. " This book attempts to place dermatology in the continuum of general medicine and to acquaint the general physician with the vast array of cutaneous diseases that indicate pathology in other organ systems."

On the whole the editors have succeeded admirably in providing an authoritative textbook, many chapters of which are outstanding. The book's weaknesses are probably partly attributable to the very large number of contributors (143), for the larger the flock the greater the risk of it including a few black sheep. The account of pityriasis lichenoides adds confusion to a confusing subject and the lack of consistency between text and legend is not helpful. The table on page 1015 contains at least 8 spelling mistakes and at least 3 factual errors. It would be easy to list other examples of haste or carelessness, but to do so would give a false impression of a book which for its considerable bulk is generally reliable.

There is a lack of balance in the amount of space devoted to some rare diseases. Fox Fordyce disease, not of great importance to the general physician, fills several pages, whilst the author of the excellent short account of Behçet's syndrome has had to confine himself to one column although the disease is a serious diagnostic and therapeutic problem. There is also some lack of balance in the approach to different topics; some chapters are diffuse and somewhat superficial, others explore the depths in thoughtful detail.

The lists of references throughout the book are an important and usually valuable feature. Only a few authors appear to have spent the last few years in complacent isolation from contemporary publications. One author dutifully cites a paper in a French-language journal, but at once destroys the reader's faith by copying an Australian mistranslation of that paper. Very few authors have failed to ensure that all statements not universally accepted are documented by an appropriate reference.

So that 2000 pages may be issued as a single volume which is not uncomfortably heavy the publishers have used a lightweight paper, which tears rather easily even if the book is not roughly treated. It is not an art paper and there is consequently some loss of definition in the illustrations, but the experiment may be regarded as highly successful for the paper is sufficiently opaque and all but a very few of the hundreds of black and white illustrations are effective and useful. There are also 32 pages of excellent illustrations in colour, printed of course on an art paper.

This book can be highly recommended.

Arthur Rook

Br. J. Derm. (1972) **87,** 551.

Quarterly Review

THERAPEUTICS XVIII

Diuretic Therapy

For many years after the discovery of the diuretic properties of organic mercurial salts (Saxl and Helig, 1920), diuresis was achieved therapeutically with drugs like mercaptomerin or mersalyl, given by injection and sometimes potentiated by ammonium chloride. Little control could be exerted over the duration or magnitude of their effect but given carefully they were effective over prolonged periods (Leff and Nussbaum, 1959). The past 15 years have produced dramatic changes and now precision in onset, duration and magnitude of diuretic activity usually can be achieved by selection of the appropriate drug and route of administration.

Diuretics may act in several ways. For example, compounds which increase renal blood flow commonly increase the urinary loss of water and electrolytes (e.g. dopamine, thaophylline and some prostaglandins). Osmotic diuretics (sucrose, mannitol and urea) produce their effects largely by an osmotic action within the renal tubule. The majority of modern diuretics, however, are drugs which reduce renal tubular sodium transport.

Most of the sodium and water normally reabsorbed from the glomerular filtrate is transferred from filtrate back to plasma by a process of active sodium transport—ion transport driven by energy-consuming mechanisms—which is a property of many renal tubular cells. While the ability to transfer sodium in this way is not unique to any particular tubular cell, three major sites can be identified where sodium transport may be modified by drugs.

In the proximal tubule about 80–85% of filtered sodium is reabsorbed, taking with it an equivalent amount of water. Secondly, in the ascending limb of the loop of Henlé, active sodium transport takes sodium into the medullary interstitial tissue where it maintains the high osmotic pressure necessary for urinary concentration processes to function normally. Thirdly, in the distal tubule, several sites of sodium reabsorption may exist but quantitatively the most important is that under the control of aldosterone, the adrenal salt-retaining hormone, which also regulates the exchange of sodium for potassium ions and thereby the renal excretion of potassium.

As sodium and water are reabsorbed by processes dependent on active sodium transport, drugs which inhibit this in any way will produce increased sodium and water loss in the urine.

Modern Diuretics

Historically, the thiadiazines (thiazides) were the first important oral diuretics (Beyer, 1958). Many modifications of their basic structure have been made which alter their rate of absorption or elimination and thereby the onset and duration of effect. Chlorothiazide, hydrochlorothiazide, hydroflumethiazide, bendrofluazide and cyclopenthiazide are all widely used. Their action is probably limited to partial inhibition of sodium transport in the distal renal tubule (Earley and Orloff, 1964). They are, therefore, not the most potent diuretics.

Later drugs within this group were the phthalimidine derivatives of which the most important representatives are chlorthalidone and clorexolone—both drugs with a prolonged effect (Douglas *et al.*, 1961; Baba *et al.*, 1966) but active at a similar site.

In 1964 a new type of highly potent oral diuretic was introduced. Frusemide, structurally related to thiazides, has proved to be of much greater potency and the magnitude of diuretic response is similar to that achieved with mersalyl (Robson *et al.*, 1964). This drug appears to act by inhibiting sodium transport in the ascending limb of the loop of Henlé and perhaps also at proximal tubular sites (Deetjen, 1966). Structurally related drugs, mefruside (Auld and Murdoch, 1971) and bumetanide (Asbury *et al.*, 1972), and one with no chemical resemblance, ethacrynic acid (Baer *et al.*, 1963), also appear to be " loop " diuretics. All of these are potent and can be used both orally and parenterally according to the urgency of the situation.

However, unwanted excessive urinary potassium loss complicates treatment with all these drugs. This is commonly countered by giving oral potassium supplements (" Slow-K ", " Kloref ") but may also be prevented by the use of an aldosterone antagonist (Spironolactone) or one of the two potassium-conserving diuretics triamterene (Baba *et al.*, 1962) or amiloride (Lant *et al.*, 1969). Spironolactone antagonizes the action of aldosterone at the distal tubule and thus promotes sodium loss and potassium retention. Triamterene, and the more potent amiloride, apparently act at the same site as spironolactone but do not antagonize aldosterone.

The use of a potent diuretic such as frusemide with a potassium-conserving drug will enhance sodium and water loss and help to maintain body potassium content.

Use of Diuretics

These compounds are used extensively in the management of patients with oedema due to congestive heart failure, nephrotic syndrome and liver disease and are also widely employed to potentiate therapy in arterial hypertension.

Apart from these clear indications, diuretics are also of value in large doses in the treatment of chronic renal failure particularly when complicated by hypertension (Rastogi *et al.*, 1971).

In diabetes insipidus, diuretics produce a paradoxical reduction in urine output which is therapeutically valuable (Crawford and Kennedy, 1959). In most centres treating patients with diabetes insipidus, long-term diuretics are preferred to the use of posterior pituitary extract and they offer the first available treatment for patients with the nephrogenic form of the disease i.e. whose kidneys are unresponsive to antidiuretic hormone.

Diuretics in Dermatology

The main indications for the use of a diuretic are the treatment of associated but unrelated medical conditions.

However, oedema is frequently part of the clinical picture of many skin disorders and is due to increased capillary permeability (Shuster and Marks, 1970). In localized lesions the oedema is of little significance and resolves with the treatment of the underlying dermatosis. In extensive erythroderma the oedema may be severe and associated with altered haemodynamics. High output cardiac failure has been observed in the condition (Shuster, 1963) and congestive cardiac failure has been described as a cause of death (Wilson, 1954; Abrahams *et al.*, 1963). The elderly and those with myocardial ischaemia are at greatest risk. Diuretics and digitalis if necessary must be given along with the treatment of the skin.

Systemic steroid therapy of erythroderma or any skin condition may itself cause oedema, due to sodium and water retention. Similarly, hypertension may also occur. As hypokalaemia is another side effect of steroid treatment the diuretic used for the control of the oedema or hypertension must be chosen with care.

Gravitational ulceration of the legs is usually associated with venous hypertension and a variable degree of oedema. The general measures taken to improve venous return—elevation of the legs, exercise and elastic support—will usually correct the oedema.

As thiazide diuretics have been shown occasionally to cause a necrotizing vasculitis (Björnberg and Gisslen, 1965) they are probably better avoided in ulcerative and purpuric conditions of the legs. However, Fox (1962) considered that diuretics might have a place in the management of chronic varicose ulceration.

There is no convincing evidence that diuretics produce any benefit in lymphoedema.

Choice of a Diuretic

Important considerations in selecting a diuretic are the efficacy, safety and cost of the treatment and the comfort of the patient. In urgent conditions such as left ventricular failure, a potent and rapidly-acting drug will be most effective and frusemide or ethacrynic acid may be given intravenously. The same treatment would be quite inappropriate for the management of mild fluid retention complicating steroid therapy where a small dose of a thiazide producing a slow diuresis might be sufficient. Elderly patients in particular do not tolerate massive diuresis and a sudden reduction in plasma volume and blood pressure may produce dizziness or blackouts in some cases.

Less seriously, many elderly patients become confused about their tablets, take their diuretics in the evening and have an uncomfortable nocturnal diuresis or even become incontinent of urine. Attention to these common-sense matters of drug administration may make a big difference to those treated with diuretics over a long period.

Many patients are kept on frusemide or ethacrynic acid for longer periods than are necessary and the aim of treatment should to be find a minimum dose of an appropriate diuretic capable of controlling fluid retention. When diuretics are used in hypertension, large doses produce little extra hypotension and small doses of thiazides in particular exert a significant effect and potentiate the action of other hypotensive drugs.

Many proprietary preparations of thiazide diuretics (often including supplementary potassium) are relatively expensive and there is little evidence that they are more effective than bendrofluazide B.P.—one of the cheapest forms of diuretic treatment.

The hazards of diuretic therapy have been mentioned already. All (except the potassium-retaining compounds amiloride, triamterene and spironolactone) increase potassium loss in the urine and cause an increase in serum uric acid levels which may lead to gout. Hyperglycaemia may also be induced.

Apart from intrinsic hazards, diuretics interact with other compounds. The action of hypotensive drugs is potentiated, as is the less well recognized reduction in blood pressure produced by tricyclic antidepressant drugs—i.e. those related to imipramine and amitryptiline. If potassium loss is not corrected, digitalis toxicity may occur early —often manifested by cardiac arrhythmias.

Recent developments in diuretic therapy have made the management of many diseases easier although these drugs do not alter the underlying aetiological condition.

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University Department of Therapeutics
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and the United Sheffield
Hospitals.

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News and Notices

Prizes of the Spanish Academy of Dermatology

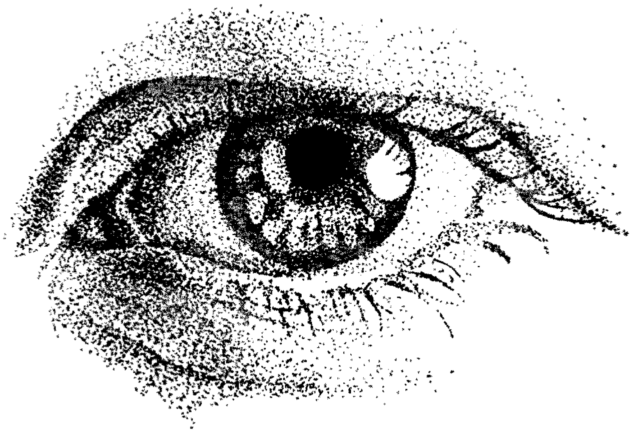
Two prizes each of 15,000 Pesetas will be awarded for original unpublished work, on any topic in dermatology or venereology, submitted by December 1st, 1972.

The Sainz de Aja prize is restricted to citizens of Spain and the Latin American countries. The Spanish Academy prize is open to dermatologists of any nationality. Further particulars may be obtained from the Spanish Academy of Dermatology, Calle Sandoval, 7, Madrid.

Appointments

Barrow and Furness and the North Lancashire and South Westmorland Groups of Hospitals

Dr David Brian Brooks has been appointed Consultant Dermatologist to the Barrow and Furness and the North Lancashire and South Westmorland Groups of Hospitals. He qualified M.B., Ch.B. (Manchester) 1958 and became M.R.C.P. in 1969. He was Resident Medical Officer in Manchester Royal Infirmary in 1964 and then became Registrar, Manchester and Salford Hospital for Skin Disease and later Registrar, Department of Dermatology, Liverpool Royal Infirmary and finally Senior Registrar, Royal Free Hospital, London. His publications include work on the Vasoconstrictor Test for Topical Corticosteroids and the Skin Flora of Infants with Napkin Rash.



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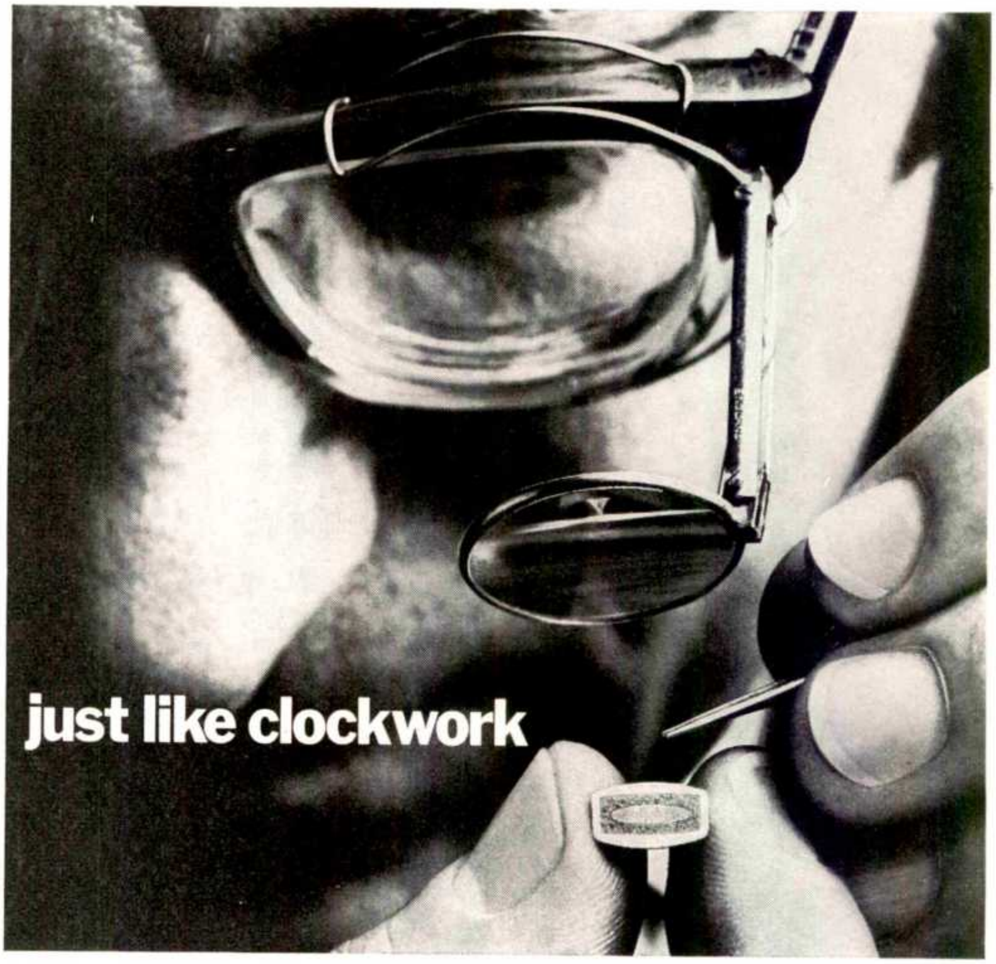

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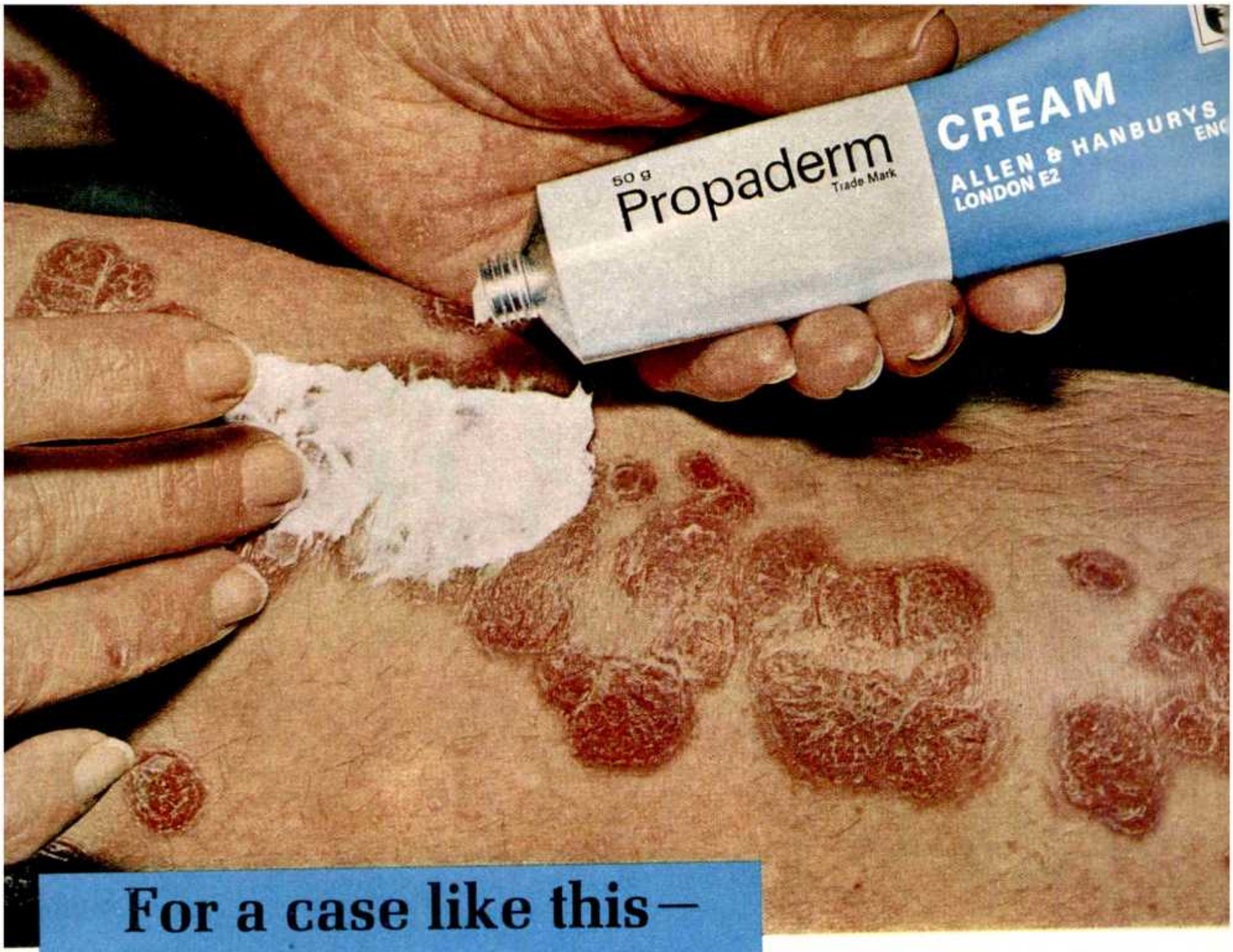

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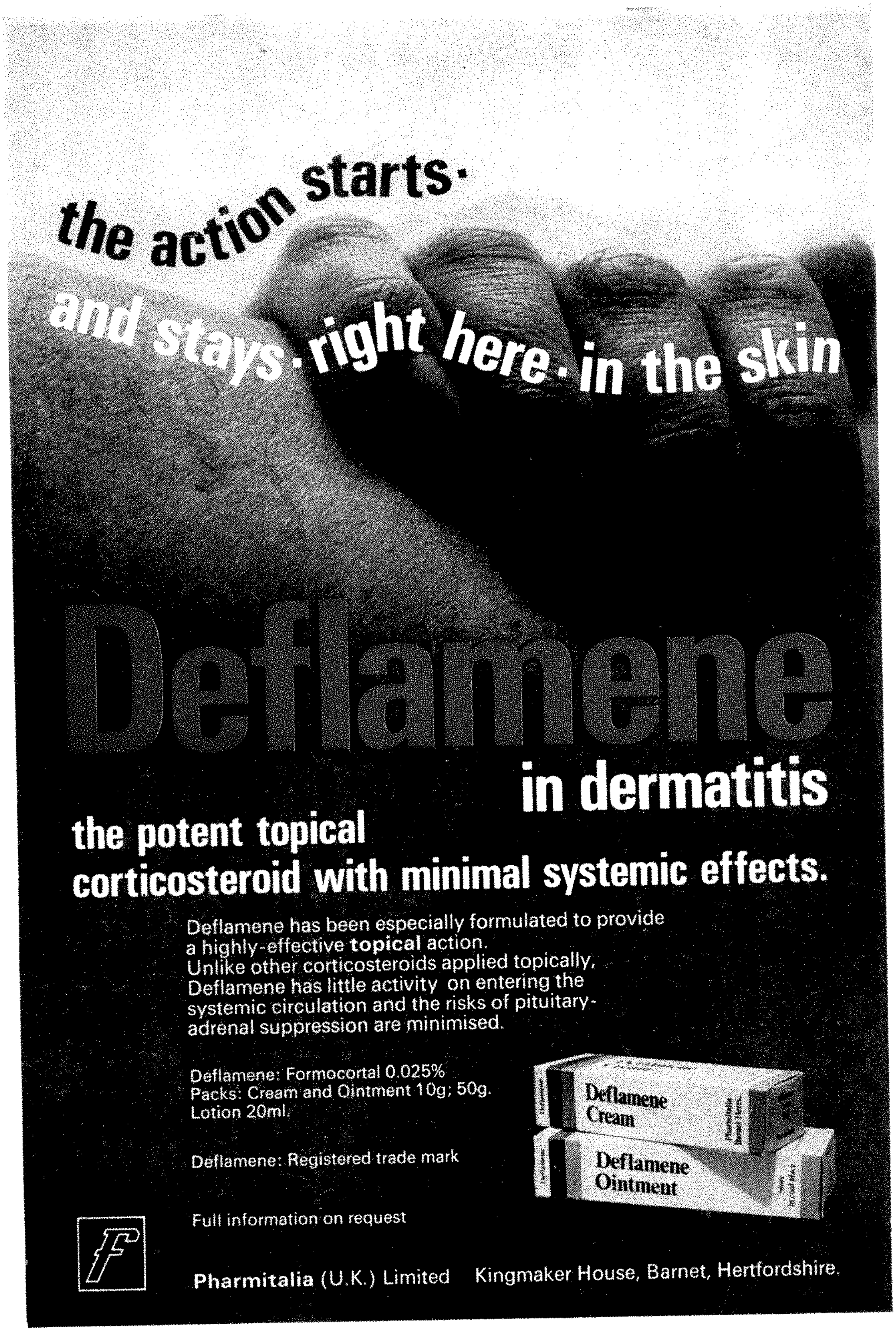

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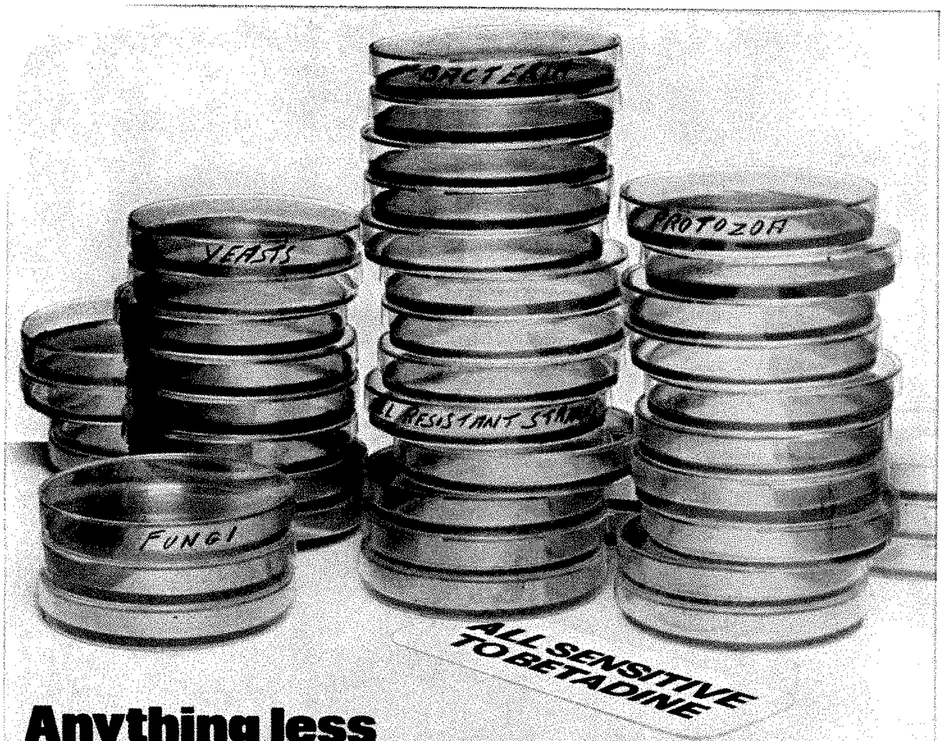

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2. Brit J Clin Pract, 1971, *25*, 322.

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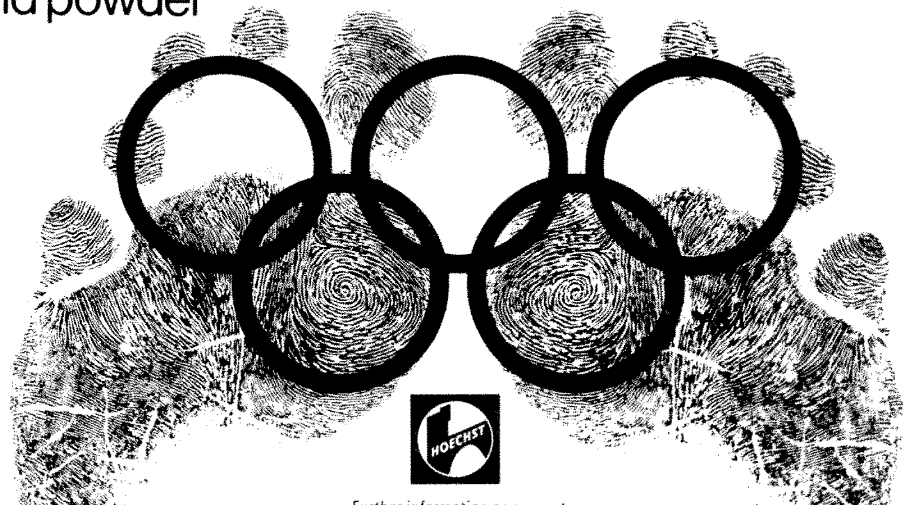

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CONTENTS (*Continued*)

History of Dermatology

Shakespeare on the Skin
J. A. COTTERILL 533

Comment

The Role of Chalones in Epidermal Homeostasis
R. MARKS 543

Books

Year Book of Dermatology (Malkinson and Pearson (Edit.)); Dermatology in General Medicine (Fitzpatrick, Arndt, Clark, Eisen, Van Scott and Vaughan (Edit.)) 549

Quarterly Review

Therapeutics XVIII. Diuretic Therapy
ANNE E. WALKER and A. J. SMITH 551

News and Notices 554

BRITISH JOURNAL OF DERMATOLOGY

7 DEC 1972

CONTENTS

PAGE

Clinical and Laboratory Investigations

Tetracycline-resistant β-Haemolytic Streptococci in Dermatological Patients.
John-Erik Brorson, Gösta Roupe and Staffan Seeberg . . . 449
The Oral Contraceptive and Variegate Porphyria
A. W. McKenzie and U. Acharya 453
Alimentary *Candida Albicans* and Napkin Rashes
Peter N. Dixon, Robert P. Warin and Mary P. English . . . 458
A Micro Method for Measuring the Carbon Dioxide Release by Small Skin Areas
F. A. J. Thiele and L. H. J. Van Kempen 463
The Selective Depigmenting Action of 8-Hydroxyquinoline on Hair Growth in the Mouse
C. E. Searle 472
The Growth of Adult Human Skin *In Vitro*
M. Levine 481
Increased *In Vitro* Radio-sensitivity of Malignant Melanoma Induced by the *In Vivo* Administration of Chlorpromazine
Michael Cooper and Yutaka Mishima 491
Correlation Between Various Proteins of Bovine Snout Epidermis
Christopher Carruthers and Malaya Bhattacharaya . . . 495
A Comparison of *In Vivo* and *In Vitro* Methods of Identifying Human Epidermal Cells in DNA Synthesis
Elizabeth Hell and Howard Maibach 506
The Quantitation of Keratinization in Human Foetal Tissues
A. Jarrett and W. Van Wyk 508
White Scalp Hairs Turning Black—an Unusual Reversal of the Ageing Process
Stanley Comaish 513
Congenital Diffuse Mottling of the Skin
D. V. Stevanović 515
Dapsone Induced Peripheral Neuropathy
Edward H. Wyatt and J. Clarke Stevens 521

Pharmacology and Treatment

Retinoic Acid in the Treatment of Psoriasis
Angus Macdonald and Lionel Fry 524
Naevus Comedonicus—Treatment with Retinoic Acid
James W. Decherd, Otto Mills and James J. Leyden . . . 528
Cosmetic Camouflage in Dermatology
T. W. Stewart and Doreen Savage 530

(*Continued on* p. iii *of cover*)

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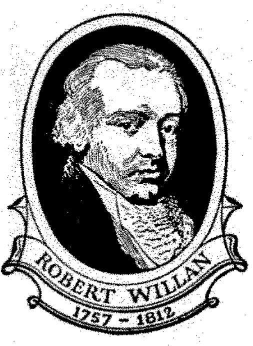

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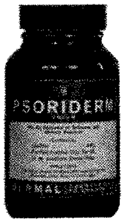

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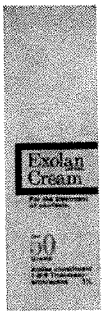

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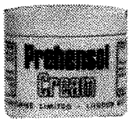

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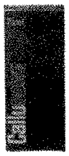

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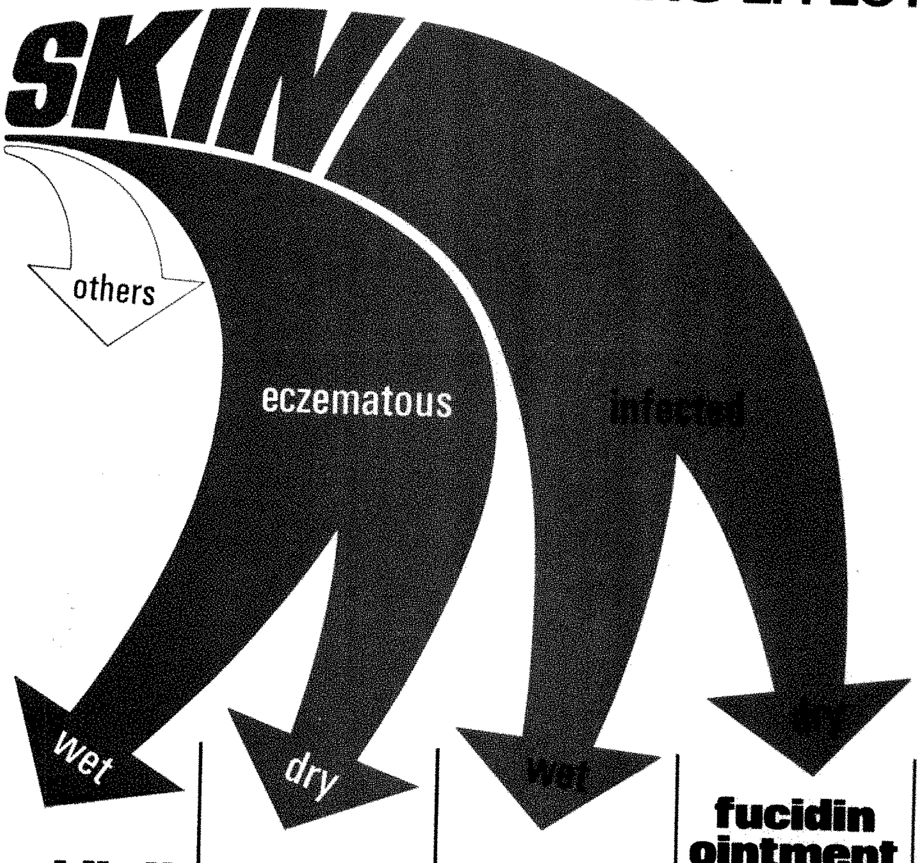

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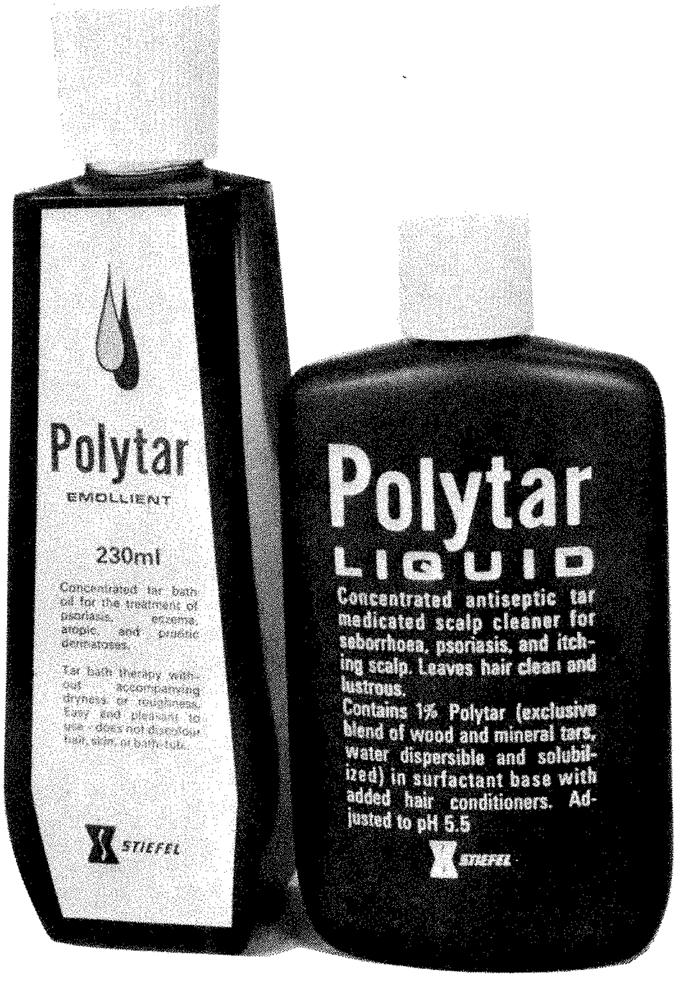

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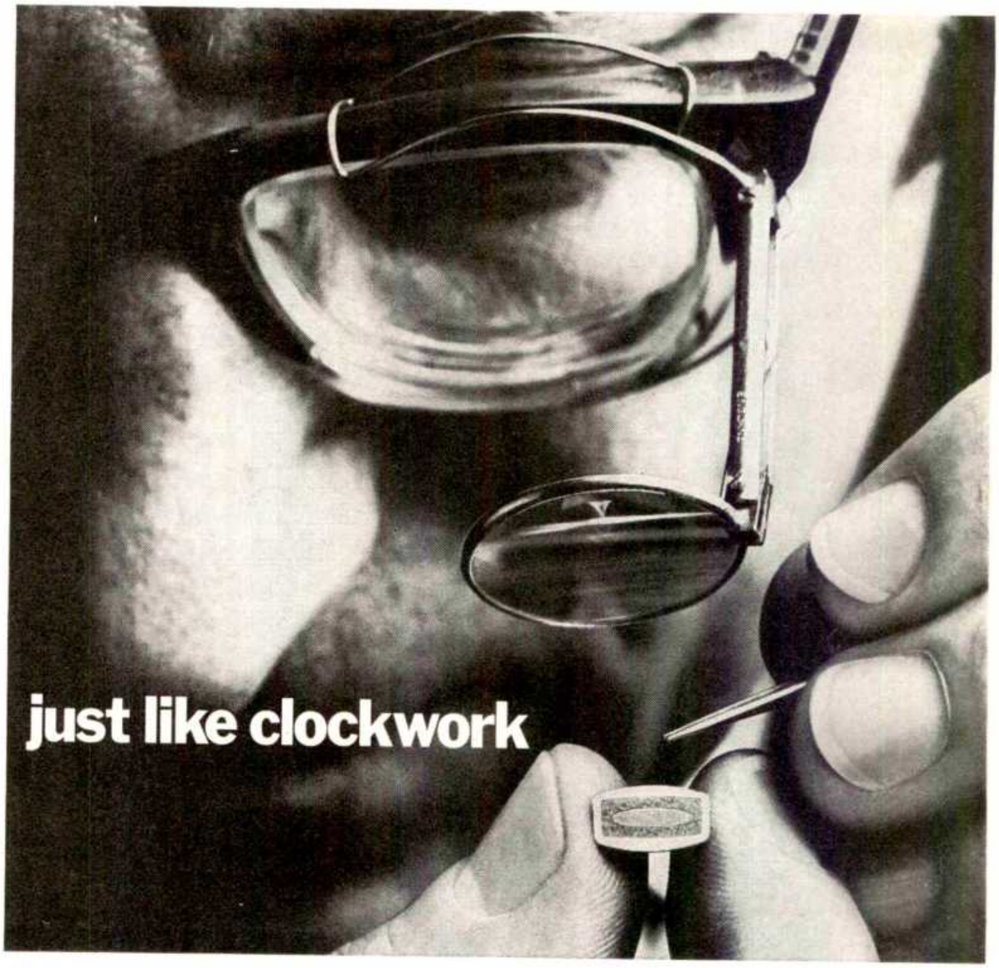

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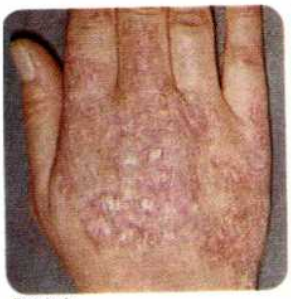
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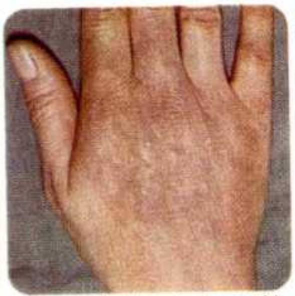
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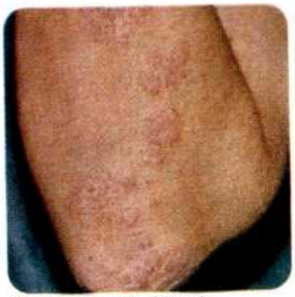
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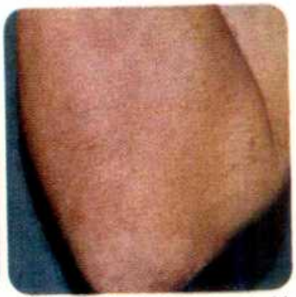
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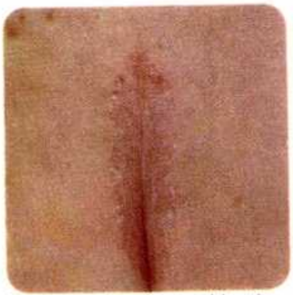
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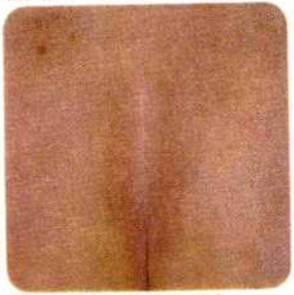
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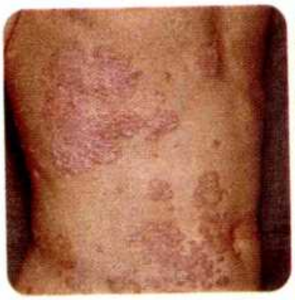
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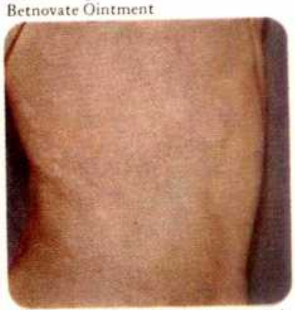
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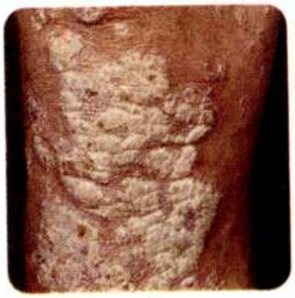
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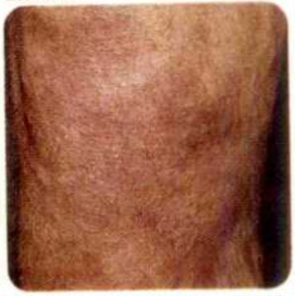
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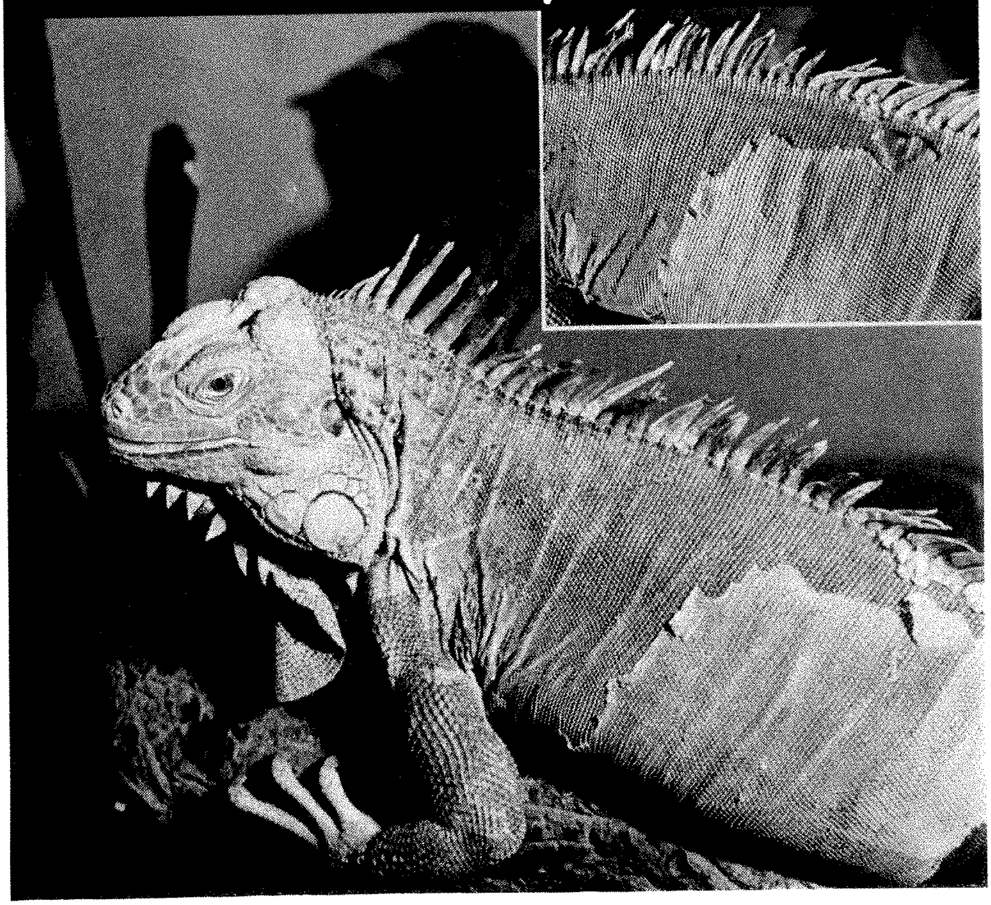

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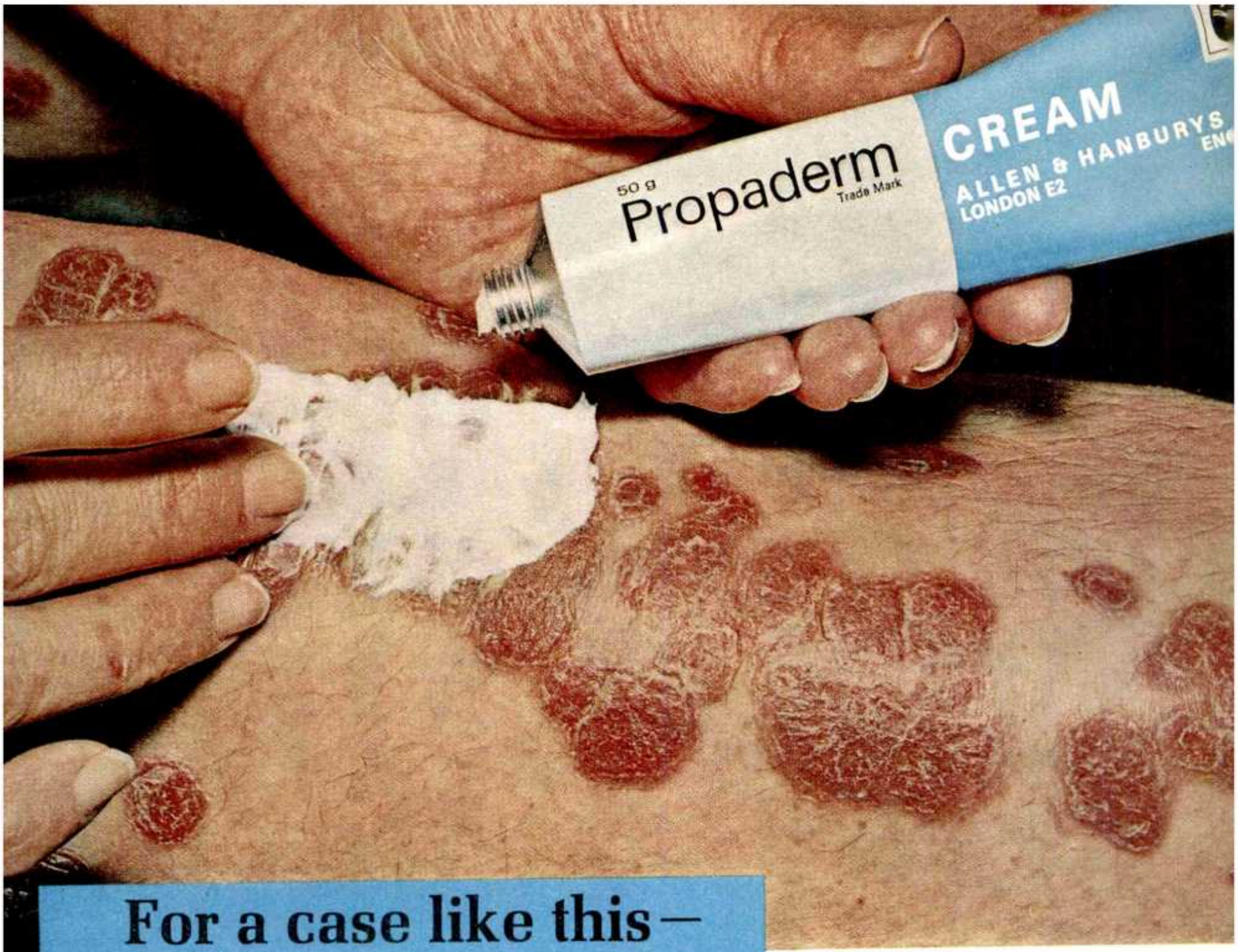

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Departments of Pathology and Dermatology, Stanford University School of Medicine, California 94305, U.S.A.

PATHOLOGY OF THE FINGER NAIL IN PSORIASIS

A CLINICOPATHOLOGICAL STUDY

KLAUS LEWIN, SHARON DeWIT AND RICHARD A. FERRINGTON

Summary.—Psoriasis of the finger nail produces metaplastic change of the nail matrix and nail bed to skin-like epithelium, following which the histological changes are essentially similar to those seen in the skin. The resulting lesions of the finger nail are of 2 types: (1) Nail plate abnormalities, the nature of which depends upon the site and the duration of disease in the matrix; (2) Nail bed abnormalities, consisting of onycholysis, subungual keratosis and splinter haemorrhages.

INVOLVEMENT of the finger nail in psoriasis is very common and has been reported in up to 50% of cases (Zaias, 1969). Although the clinical manifestations have been well described (Samman 1965), there has been a notable paucity of histopathological studies (Alkiewicz, 1948; Zaias, 1969) because of the difficulty in obtaining biopsy material. Recently Zaias (1967) has shown that material can be obtained by biopsy without disfigurement of the finger nail. Using this technique, we have studied the common lesions of psoriasis of the finger nail.

SELECTION OF MATERIAL AND METHODS

Seven cases were studied, one of them post-mortem. They were chosen because they showed the different nail deformities commonly found in psoriasis. The biopsy specimens consisted of longitudinal strips of nail and nail bed, and were obtained by the method described by Zaias (1967). The material was fixed in 10% formol saline for 2 days. The nail plate was then softened in 3% phenol from 2 to 7 days. This was followed by routine processing and embedding in paraffin wax. Sections were cut at 5 μm and stained with H. and E., gram, Gomori's methenamine silver stain and elastic van Gieson.

RESULTS

The Normal Nail

Although the normal histology and growth pattern are well described in the literature (Pinkus, 1927; Lewis, 1954; Lewin, 1965), it is helpful to review them briefly in order to understand the pathological changes occurring in psoriasis.

The finger nail is made up of nail plate, nail bed and matrix and the nail folds. The nail bed is structurally similar to skin, except that the epidermal layer is devoid of stratum lucidum and stratum granulosum and lacks secretory and

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Read before the International Symposium on Psoriasis, Stanford University, Palo Alto, California, July 10, 1971.

pilosebaceous adnexae. The dermis of the nail bed is arranged in longitudinal grooves and ridges, and into the former project the rete ridges, which are normally thin, fine structures. The boundary between the epidermis and dermis of the nail bed is, therefore, serrated in a transverse section (Fig. 1), but flat in longitudinal planes. The grooves and ridges become blunted proximally, in the nail matrix, which is the area of nail roughly corresponding to the lunula, including the part covered by the posterior nail fold (Fig. 2).

The epidermis of the nail matrix is particularly thick and passes gradually into the substance of the nail, the formation of which takes place mainly in this region by a series of changes, broadly comparable to keratinization of the epidermis—

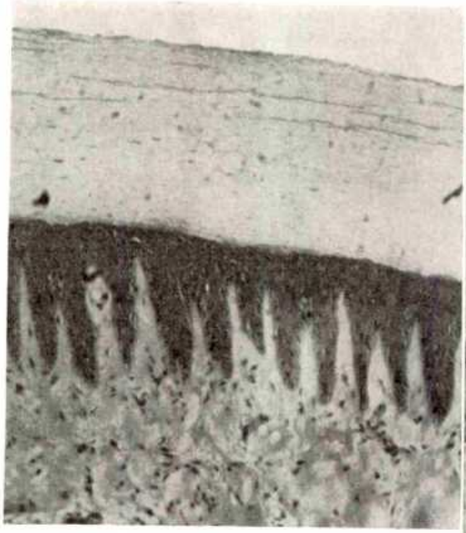

FIG. 1.—The normal finger nail. Transverse section (T.S.), showing nail plate in close apposition to the serrated epithelium of nail bed which lacks a stratum lucidum and granulosum. Also note absence of secretory and pilosebaceous adnexae. H. and E. × 100.

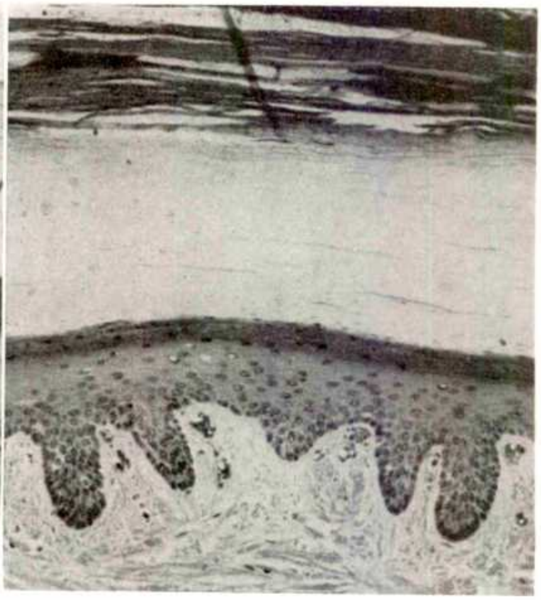

FIG. 2.—The normal nail matrix. The rete ridges have given way to blunted prominences. Nail formation occurs by a process of nucleolysis and cell shrinkage. T.S.; H. and E. × 250.

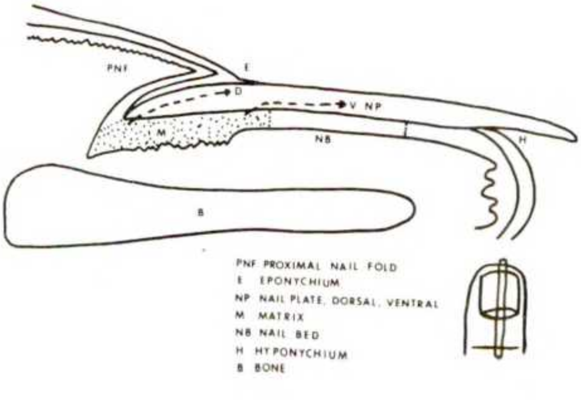

FIG. 3.—Growth of normal nail; longitudinal section.

swelling of the cell, nucleolysis and subsequent shrinkage (Fig. 2). At the free edge of the nail the usual horny layer begins again, and is called the hyponychium.

The nail plate arises from the entire length of the nail matrix, the proximal portion of which forms the upper-most layer, and the distal half, the deeper layers (Fig. 3).

The nail folds surround the proximal and lateral edges of the nail plate and have the structure of skin.

The Psoriatic Nail

Classification.—The nail abnormalities were classified on clinical criteria into acute and chronic. The acute psoriatic nail showed intense inflammation of the terminal phalanx and deformity or absence of the whole nail plate. In the chronic psoriatic nail there was only partial involvement of the finger nail, with no significant inflammation of the terminal phalanx. Arthritis of the terminal interphalangeal joint (T.I.P.) was an inconstant finding, but, when it occurred, was more severe in the acute cases.

Acute psoriasis of the finger nail.—There were 3 patients in this group, 2 of whom showed severe reddening of the terminal phalanges, arthritis of the T.I.P. joints and dystrophic nail plates. The nail lesions in one patient, who died of fulminating exfoliative dermatitis and septicaemia, were continuous with the exfoliative process of the skin. The nail plate was absent and replaced by a lightly adherent, semilucent, keratinous crust. The nail plates of the other 2 patients, both of whom had longstanding psoriasis of the finger nail, were replaced by hyperkeratotic scales firmly adherent to the nail bed and matrix. One of the latter had also superficial pustular areas in the thickened nail bed.

On microscopy the changes in the finger nail bed and matrix were similar to those found in severe psoriasis of the skin. There was marked acanthosis, elongation of epidermal ridges and severe hyperkeratosis (Fig. 4). In places the epidermis was drawn downwards to produce a rather characteristic funnel-shaped deformity (Fig. 5). Many of the epidermal cells showed marked intranuclear and cytoplasmic vacuolation. There was a heavy inflammatory infiltrate of the dermis, consisting of many polymorphs and lesser numbers of lymphocytes and

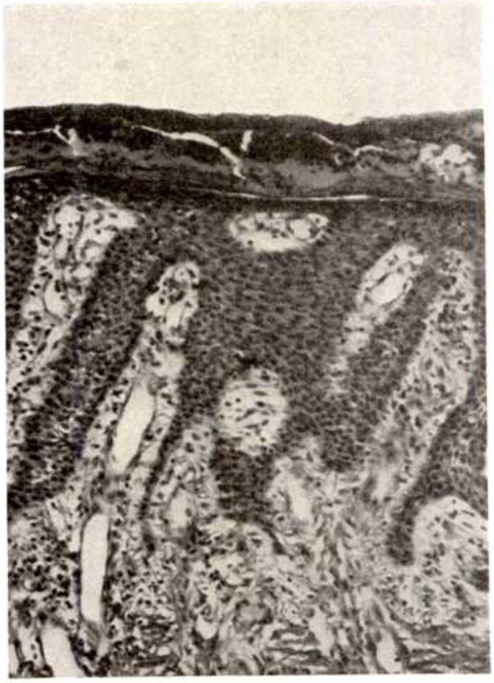

FIG. 4.—Acute fulminating psoriasis, showing parakeratosis, elongation of rete ridges, thinning or suprapapillary portions of epidermis, prominence of dermal papillae and dilatation of dermal capillaries. Also note Munro's micro-abscesses. T.S.; H. and E. × 400.

plasma cells. The polymorphs showed extensive migration into the epidermis, often forming Munro's micro-abscesses (Fig. 4), and, in one patient, producing the histological features of pustular psoriasis. Capillaries in the dermal papillae were dilated and tortuous, and some had ruptured and bled either into the epidermis or the hyperkeratotic layer, or in between the two. The nail matrix showed no normal nail plate formation. Instead, it gave rise to a thickened, hyperkeratotic layer. In the patient with exfoliative psoriasis the hyperkeratotic layer consisted of a loosely adherent, parakeratotic scale (Fig. 5). In the other 2 patients the hyperkeratotic tissue consisted of alternating layers of parakeratosis and orthokeratosis which were related to the presence or absence of the granular layer (Fig. 6), the granular layer being covered by orthokeratotic cells and the agranular epithelium giving rise to the parakeratotic cells. The posterior nail fold showed severe atrophy (Fig. 6) but otherwise the same changes as already described.

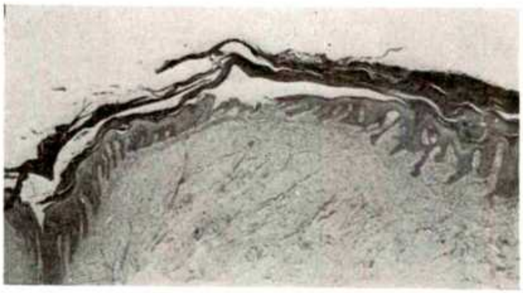

FIG. 5.—Acute fulminating psoriasis (Case 1). Low power magnification, showing loosely adherent hyperkeratotic tissue. Note also the atrophy of the posterior nail fold on the left, and the downward extension of the squamous epithelium of the nail bed on the right. L.S.; H. and E. × 6.

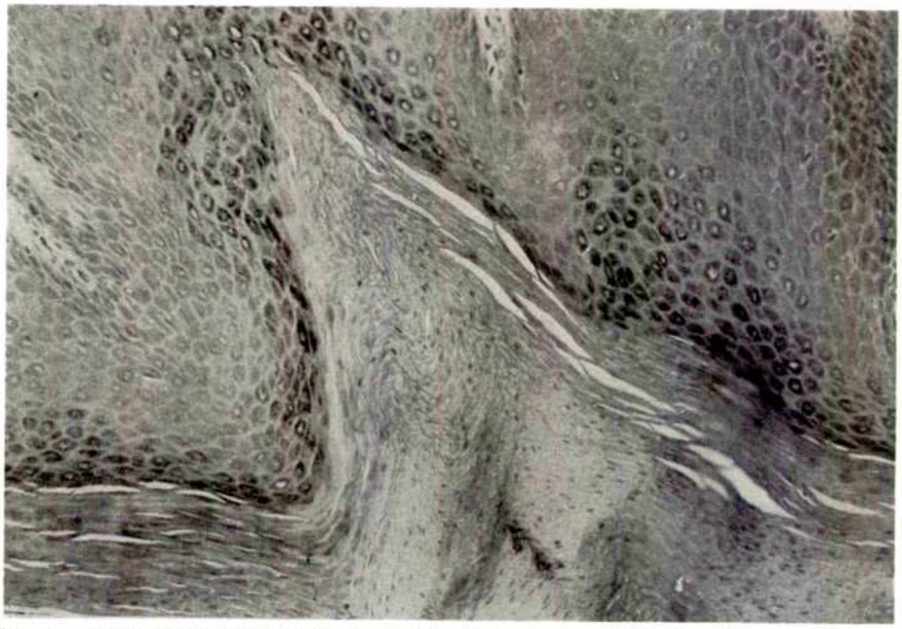

FIG. 6.—Acute psoriasis (Case 2), showing higher power magnification of nail matrix and posterior nail fold. Note prominent granular layer giving rise to orthokeratotic tissue and atrophy of posterior nail fold. L.S.; H. and E. × 400.

Organisms were found in the hyperkeratotic tissue in one case. They consisted predominantly of gram-positive cocci and occasional gram-negative rods.

Chronic psoriasis of the finger nail.—Biopsies from 4 patients were examined. In all cases the histological changes were similar to, but less severe than, those in the acute phase, and the nail involvement was focal. In one instance the lesion was confined to the nail bed; but, in the 3 other cases, there was evidence of co-existing nail bed and nail matrix disease. The following lesions of chronic psoriasis, some of which were found together, were examined and are described in greater detail:

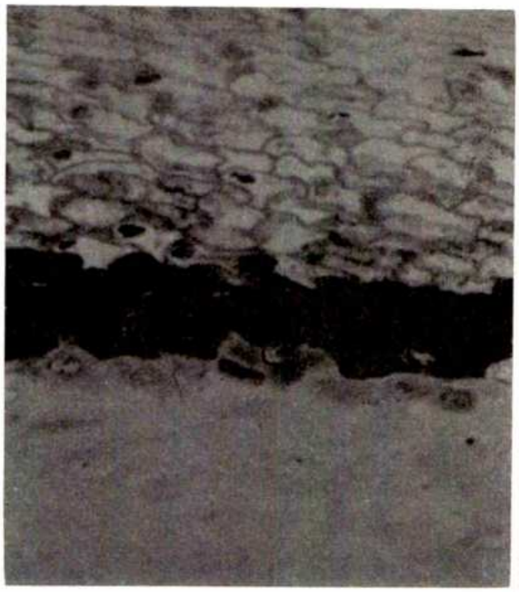

FIG. 7.—Nail matrix in chronic psoriasis, showing abnormal nucleolysis and shrinkage of epidermal cells. Note prominent mosaic pattern of residual epidermal cells in nail plate. Gram. × 400.

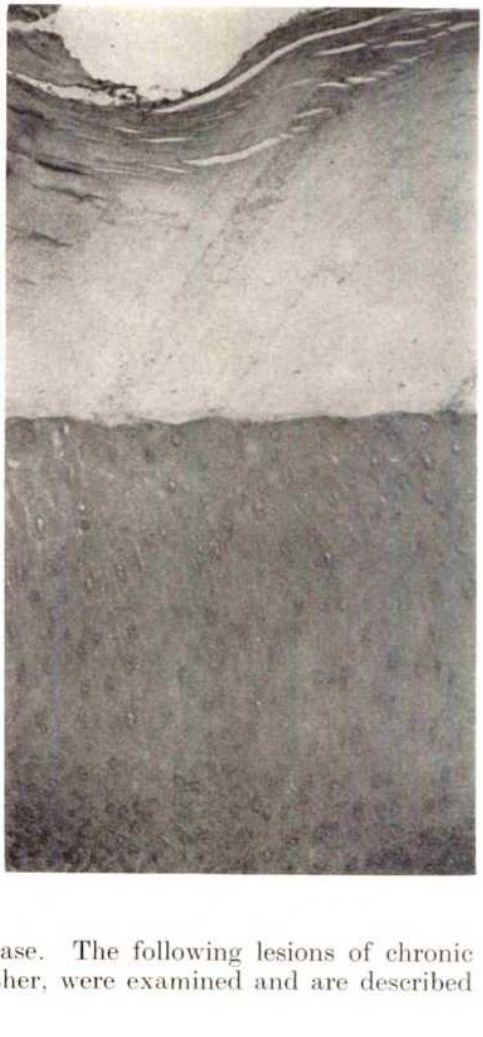

FIG. 8.—Finger nail pitting (Case 7). Nail plate lamellae show fissuring. Note also the residual parakeratotic cells superficially. L.S.; H. and E. × 100.

(1) *Nail plate abnormalities*

Nail pitting—2 of 4 cases
Longitudinal fissuring—1 of 4 cases
Transverse fissuring—1 of 4 cases
Abnormal epidermal condensation in nail matrix—4 of 4 cases

(2) *Nail bed abnormalities*

Subungual keratosis and onycholysis—3 of 4 cases
Bacterial and fungal infection—4 of 4 cases

Nail Plate Abnormalities

Abnormal condensation of epidermis of nail matrix.—All 4 cases of chronic psoriasis showed minor degrees of this abnormality. The nail matrix was moderately thickened and rarely inflamed. The normal sharp line of demarcation, between epidermis and nail plate, was replaced by an irregular wavy or rolled margin. There was abnormal condensation of epidermal cells which, instead of their normal transparent appearance, retained variable amounts of eosinophilic cytoplasm and occasionally also their nuclei (Fig. 7).

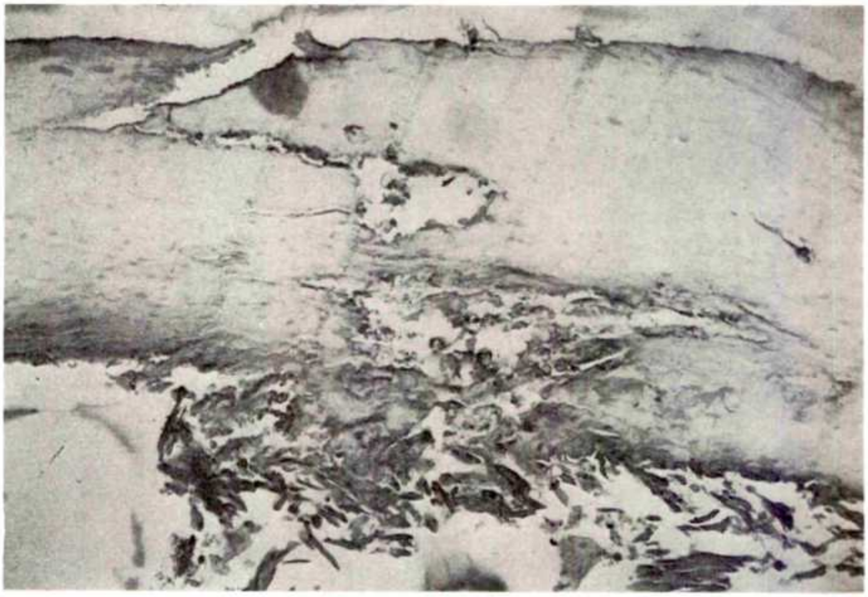

FIG. 9.—Section of finger nail, showing transverse fissuring (Case 7). Full thickness defect of nail plate has caused distal half to break off. More proximally, clefts can be seen in the centre of the nail plate. Note also subungual hyperkeratosis. L.S.; H. and E. × 5.

Pits.—These were small, usually round, superficial defects of the nail plate. They were lined by parakeratotic cells, loosely adherent to one another (Fig. 8).

Longitudinal ridging.—The nail plate was defective throughout its length, but not uniformly abnormal. The distal portion of the nail, in its middle and deeper parts, showed replacement of the normal tissue by crumbly parakeratotic material (Fig. 9). The nail bed showed acanthosis and hyperkeratosis. Only a tiny piece of nail matrix was examined, and this showed a microscopic focus of chronic lymphocytic infiltration and acanthosis.

Transverse fissuring.—Apart from a thin superficial parakeratotic layer, there was a full thickness defect of the nail plate, which had caused the distal half to break off. More proximally, clefts were present in the centre of the nail plate composed of parakeratotic cells, and one pit was also seen (Fig. 10). The nail matrix showed mild acanthosis, but no active inflammation. The nail bed showed moderate hyperkeratosis.

Abnormality of the Nail Bed and Hyponychium

Onycholysis and subungual hyperkeratosis.—All 4 cases of chronic psoriasis of the nail showed these changes. There was hyperkeratosis of the nail bed and consequent loosening and lifting of the nail plate from the underlying epithielum (Fig. 11). The squamous epithelium was moderately thickened and in places showed the characteristic downward dipping already described. Inflammation and vascular dilatation were not prominent features.

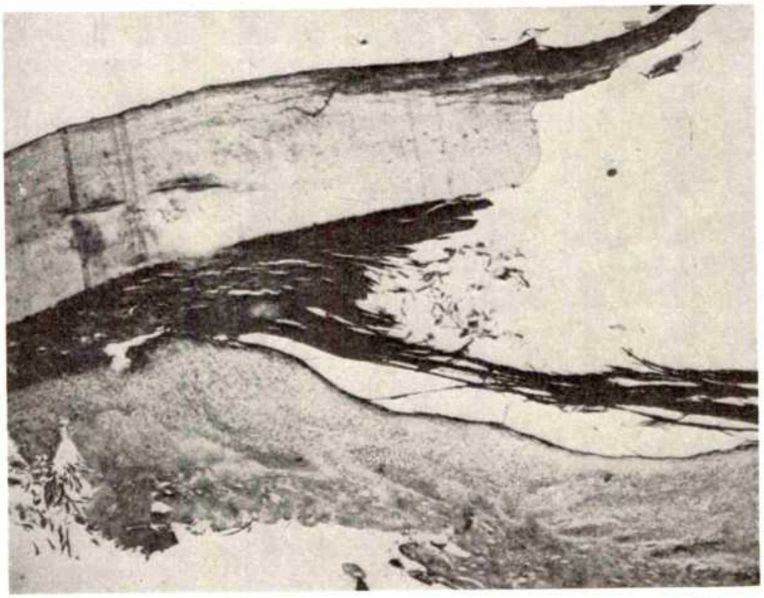

FIG. 10.—Section of finger nail, showing longitudinal fissuring. Note crumbly nail plate partly replaced by parakeratotic material. L.S.; H. and E. × 10.

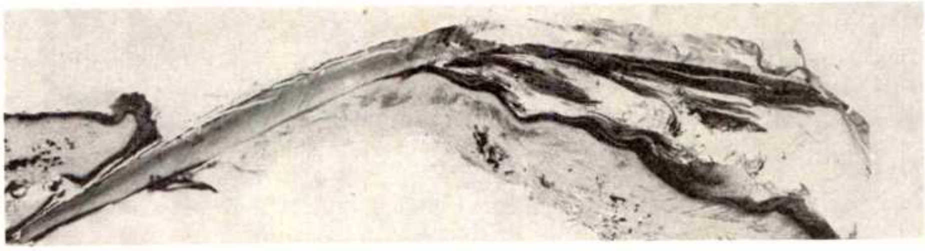

FIG. 11.—Chronic psoriasis with onycholysis (Case 4), showing marked subungual keratosis and lifting of nail plate. L.S.; H. and E. × 8.

Infection with organisms.—Organisms were found in all 4 cases. They formed colonies within the hyperkeratotic tissue and abnormal nail plate. The organisms consisted predominantly of gram-positive cocci, occasional gram-negative rods and in 2 cases fungi, consistent with monilia.

DISCUSSION

Although the pathological changes of psoriasis of the finger nail show many of the changes found in the skin, there are several unusual and interesting features. One of the most striking changes occurs in the nail bed and nail matrix, which differ histologically from the skin in that they do not normally have a granular layer. In acute psoriasis they develop this layer and then look like the squamous epithelium of the skin. Once this change has occurred the nail bed and matrix behave like skin. The granular layer may recede or even disappear in severe or fulminating disease. The stratum corneum frequently shows alternating layers of orthokeratosis and parakeratosis, which are related to the presence or absence of the stratum granulosum, the stratum granulosum giving rise to non-nucleated cells and the non-granular epithelium giving rise to nucleated cells. Similar changes have been described in psoriasis of the skin and have been ascribed to increased epithelial turnover and intracellular oedema causing an alteration in the normal process of cornification (Gans, 1952). The above changes, however, are not specific for psoriasis and occur in any skin condition in which there is marked acanthosis (Lever, 1961). Thus, as a general rule, wherever there is parakeratosis, the granular layer is absent and intracellular oedema pronounced.

Since the nail matrix gives rise to the nail plate by condensation of the superficial epidermal cells, it follows that any disease which interferes with this process will give rise to nail plate abnormalities.

The most characteristic change in psoriasis is the incorporation of parakeratotic scales, which are crumbly and poorly adherent to one another, into the substance of the nail plate. The resulting nail abnormality will depend on the extent of the nail matrix involvement. If the entire nail matrix is diseased, no normal nail will be formed; instead, it is replaced by a hyperkeratotic crust, as in the acute cases. If only part of the nail matrix is involved, longitudinal or transverse defects such as ridges or depressions will be produced.

The nature of the nail abnormality will depend also upon which portion of the matrix is involved. Disease of the proximal part of the matrix, which forms the superficial layers of the nail plate, will produce defects such as pitting and superficial depressions. This is because the parakeratotic cells, which are incorporated into the nail plate, are poorly adherent to one another and easily dislodged by minor trauma. Disease of the distal part of the nail matrix, which forms the deeper portions of the nail plate, produces leuconychia due to loss of transparency of the nail plate. The appearance of these lesions is said to be due to numerous air fissures separating the hyperkeratotic scales, as in the skin (Alkiewicz, 1948).

Psoriasis is a disease of exacerbations and remissions. Since it takes approximately 3 months for a nail to grow from matrix to hyponychium, psoriasis of the finger nail affords a convenient time lapse study of this disease. Although the matrix may be normal at the time of examination, lesions such as pits, grooves and furrows and leuconychia reflect previous disease of the nail matrix. Of interest in all 4 cases of chronic psoriasis is the finding in the nail plate of abnormal condensation of epidermal cells which retain variable amounts of eosinophilic cytoplasm and occasional nuclei. To some degree this occurs in the normal nail (Lewin, 1965); however, in psoriasis, this is markedly accentuated and possibly contributes to the thickening and yellow discoloration of the nail.

Disease of the nail bed spares the nail plate and produces a different set of abnormalities. Thus, hyperkeratosis causes either oncholysis, by loosening the nail plate from the nail bed and lifting it up, or subungual keratosis of the hyponychium. The onycholytic space, which is filled with keratin, forms an excellent culture medium for bacteria and monilia, which were found in 6 of our 7 cases. Fungal dermatophytes, which readily infect the nail, were not seen. Zaias (1969) thinks this is due to serum-like glycoprotein exudates in the psoriatic nail which are inhibitory to dermatophytes but not to yeasts.

Haemorrhage is common, occurring mainly into the subungual space to produce splinter haemorrhage. However, it occurs occasionally in the nail matrix and is then incorporated into the nail plate or hyperkeratotic layer.

We gratefully acknowledge the help of Dr David Korn and Dr Richard Kempson in reviewing the manuscript and for offering helpful suggestions. We would also like to thank Dr Eugene Farber for his encouragement and help.

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FURTHER EXPERIENCES OF TOXIC EPIDERMAL NECROLYSIS INCRIMINATING ALLOPURINOL, PYRAZOLONE AND DERIVATIVES

J. D. STRATIGOS, ST. K. BARTSOKAS* AND J. CAPETANAKIS

Summary.—Four cases of toxic epidermal necrolysis, confirmed by skin biopsy, are described. The first case was due to allopurinol. The second case was caused by a pyrazolone derivative in a patient who, 15 days previously, had developed an urticarial exanthem from tetanus antitoxin. In these 2 fatal cases Stevens–Johnson syndrome coexisted. In the remaining cases toxic epidermal necrolysis occurred alone and recovery was uneventful.

IN the aetiology of toxic epidermal necrolysis (TEN) many factors have been incriminated (Lowney *et al.*, 1967; Lyell, 1967). The pathogenetic mechanism of this syndrome of multiple aetiology is obscure, and more clinical and laboratory observations are needed for its solution.

Among drugs evoking TEN, allopurinol has recently been reported (Kantor, 1970). Another interesting clinical observation has been that TEN can be evoked by drugs of 2 different groups acting together (Braun-Falco, 1970).

The purpose of this communication is to report first, a second case of TEN due to allopurinol; secondly, a case in which the patient developed drug-induced TEN and who had shortly before had urticaria from tetanus antitoxin; thirdly, to support the impression that association of TEN and Stevens–Johnson syndrome occurs in the group of cases probably due to drugs (Wilkinson, 1971), and has a serious prognosis.

CASE REPORTS

Case 1.—A woman aged 58. For many years she had received rauwolfia for essential hypertension. The serum uric acid was elevated and allopurinol 600 mg daily was prescribed. Two days later she developed erythema multiforme, for which she was admitted to hospital. On examination she was desperately ill and febrile. Treatment with prednisone 200 mg daily was started. No pathogenic bacteria were isolated from repeated cultures of bullae, blood and urine. Routine laboratory examinations were normal. The skin biopsy showed typical lesions of TEN. During the following days the rash became more characteristic of the Stevens–Johnson syndrome. By the fifth day, toxic epidermal necrolysis was obviously present (Fig. 1), and mild intestinal bleeding developed; this subsequently stopped. On the seventh day *Proteus vulgaris* was recovered from the urine, and chloramphenicol 2 g daily was started. Denuded skin areas were treated locally with an aqueous solution of 0·5% silver nitrate. Fluids and electrolytes maintained the renal function. Prednisone was decreased gradually and, although her general and local conditions improved, respiratory symptoms devel-

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oped on the eleventh day, without signs of pulmonary infection, and the patient died on the twelfth day of the illness.

Case 2.—A woman aged 38. Following ablation of an ovarian cyst, she received an intravenous injection of a pyrazolone derivative. A few hours later a rash appeared. This spread to the mucosa of the oral cavity and vagina and to the fingers. On the second day the rash became transformed into the Stevens–Johnson type of erythema multiforme, and by the third day a toxic epidermal necrolysis (Fig. 2) as confirmed by skin biopsy had covered 60% of her body. Fifteen days before the operation she had received tetanus antitoxin, which was followed by an urticarial exanthem lasting 3 days. No antibiotics were given before, during or after the operation. Laboratory findings were normal. On the second day 200 mg prednisone daily was started. Until the fifth day repeated cultures of blood, bullae and urine were sterile, but *Pseudomonas aeruginosa* was then recovered from the urine. Carbenicillin was given, while prednisone was continued at the same dose. On the ensuing days her general and local conditions deteriorated and, although fluid and electrolyte balance was preserved, oliguria occurred on the seventh day and the patient died 3 days later.

Cases 3 *and* 4.—A man aged 80 and a woman aged 26 had received respectively, for a mild upper respiratory infection, a common preparation containing aspirin, caffeine and aminophenazone, and a pyrazolone derivative.

Twenty-four hours later toxic epidermal necrolysis appeared in the first patient and pursued a mild course. The second patient, a few hours after taking the pills, started to develop exfoliation of the skin which, on the sixth day, began to improve in the usual manner. Both cases were verified by the histological findings of skin biopsies. In both patients corticosteroids and antibiotics were given, while denuded skin areas were kept sterile with local application of a 0·5% aqueous solution of silver nitrate (Izumi *et al.*, 1970).

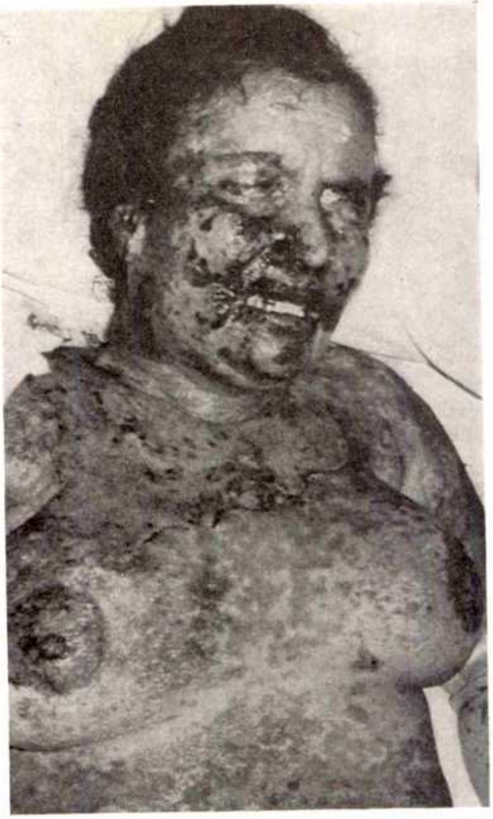

FIG. 1.—Case 1 showing combination of toxic epidermal necrolysis and Stevens–Johnson syndrome.

DISCUSSION

In our first case, toxic epidermal necrolysis started 2 days after allopurinol was prescribed. There was no indication of any previous bacterial or viral disease, or of allergic reaction. Although on the seventh day *Proteus vulgaris* was found in the urine, kidney function was well preserved until death. In another case, however, in which toxic epidermal necrolysis was due to allopurinol (Kantor, 1970), the patient died from renal failure, sepsis and pneumonia, and the autopsy findings suggested that the skin damage led to a release of a basement membrane antigen, which resulted in the formation of a cross-reacting antibody with the glomerular basement membrane. Our patient died from acute respiratory failure. Could a similar antigen–antibody system, cross-reacting with pulmonary basement membrane, be incriminated?

In the second case post-operative administration of a pyrazolone derivative evoked the syndrome. Fifteen days before the operation, shortly after she had received tetanus antitoxin, an urticaria was noticed.

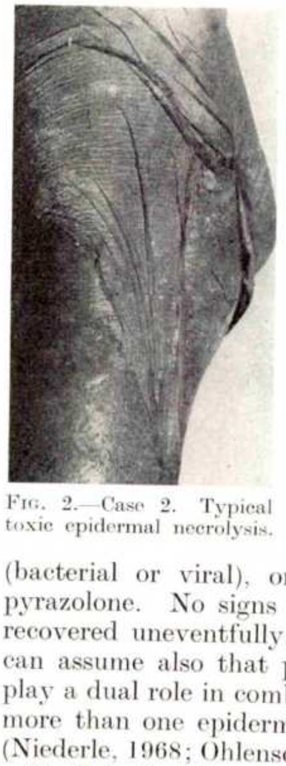

FIG. 2.—Case 2. Typical toxic epidermal necrolysis.

In a recent symposium on TEN (Hoffman, 1970) there is described the case of a woman who 4 months previously had been treated with sulphonamides and penicillin, and who had developed toxic epidermal necrolysis. After the administration of a common preparation for a venous disease, she developed a new attack, with a fatal outcome.

Our second case also had a known cause. It is highly probable that sensitivity to the pyrazolone developed in a predisposed person, as is indicated by the earlier reaction to tetanus antitoxin. This supports the idea that TEN can be evoked not only by the combination of different groups of drugs but also by the combination of drugs and other substances.

In both Cases 1 and 2 toxic epidermal necrolysis developed after drugs were administered and Stevens–Johnson coexisted, a feature which has been already recognized as a bad prognostic sign.

By contrast, in the other 2 milder cases, the syndrome could be due to an upper respiratory infection (bacterial or viral), or in combination with other substances, in these cases pyrazolone. No signs of erythema multiforme were present, and both patients recovered uneventfully. By virtue of the absence of erythema multiforme we can assume also that pyrazolone and its derivatives, like sulphonamides, could play a dual role in combination with certain drugs and are capable of producing more than one epidermolytic process, manifest in at least 2 forms with TEN (Niederle, 1968; Ohlenschlaeger, 1966).

Until further data regarding the prescribing of pyrazolone and derivatives can be accumulated, and in view of the fact that bacteriophage 71 (staphylococcus) has been isolated in certain cases of TEN (Arbuthnott, 1970), it is also possible that Cases 3 and 4 could be due primarily to the upper respiratory infection.

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ALLERGIC CONTACT DERMATITIS FROM PYRETHRUM (*CHRYSANTHEMUM* SPP.)

THE ROLES OF PYRETHROSIN, A SESQUITERPENE LACTONE, AND OF PYRETHRIN II

JOHN C. MITCHELL, GILLES DUPUIS AND G. H. NEIL TOWERS

Summary.—In a patient with allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp., family Compositae), pyrethrosin produced strong, and pyrethrin II weak, positive patch test responses.

Some other compounds derived from pyrethrum, viz. chrysanthemumic acid, the ethyl ester of this acid and its naturally occurring insecticidal esters, pyrethrin I, cinerin I and II, jasmolin I and II, and the synthetic ester allethrin and chrysanthemin, taraxasterol (pyrethrol) and tiglic acid produced negative patch test responses.

In this patient, pyrethrosin, a sesquiterpene lactone which does not have insecticidal properties, was the principal allergenic fraction of pyrethrum derived from species of *Chrysanthemum*. Of the 8 esters of chrysanthemumic acid which were tested, pyrethrin II was also allergenic in this case.

Pyrethrum present in insect powder and in scabiecidal and anti-mosquito preparations has been reported to cause allergic eczematous contact dermatitis (McCord *et al.*, 1921; Sulzberger and Weinberg, 1930; Sequeira, 1936; Editorial, *Br. med. J.*, 1936; Sweitzer, 1936; Martin and Hester, 1941; Lord and Johnson, 1947; Rook, 1960; McCord, 1962; Mitchell, 1969).

A review of the compounds known to be present in pyrethrum powder, which is derived from species of *Chrysanthemum*, was prompted by reports of the role of sesquiterpene lactones derived from members of Compositae in the causation of such dermatitis (Mitchell, 1969; Mitchell *et al.*, 1970; Mitchell *et al.*, 1971; Mitchell and Dupuis, 1971).

Pyrethrum, also named Dalmatian or Persian insect powder, consists of the dried flower heads of *Chrysanthemum cinerariaefolium* Vis. and of *C. coccineum* Willd. (Tribe Anthemidae, Family Compositae: common names, Dalmatian Chrysanthemum, Japanese Daisy, Painted Daisy).

These plants contain chrysanthemumic acid and the naturally occurring esters of this acid, viz. pyrethrin, cinerin and jasmolin (Head, 1966). Synthetic esters of chrysanthemumic acid, e.g. allethrin, are prepared commercially as insecticides.

Among the chemical compounds present in pyrethrum powder is pyrethrosin, a sesquiterpene lactone, one of some 200 such compounds which have previously been reported for allergic contact dermatitis (Mitchell and Dupuis, 1971).

Pyrethrosin and pyrethrum, and also some other derivatives of pyrethrum, were therefore tested for allergenic activity.

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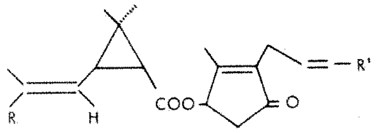
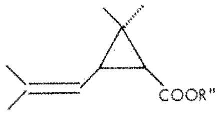


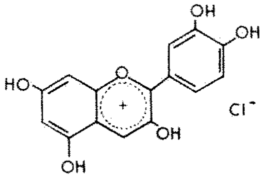
I R, CH_3; R', CH:CH_2 PYRETHRIN I
II R, CH_3; R', CH_3 CINERIN I
III R, CH_3; R', CH_2CH_3 JASMOLIN I
IV R, $COOCH_3$; R', CH:CH_2 PYRETHRIN II

V R, $COOCH_3$; R', CH_3 CINERIN II
VI R, $COOCH_3$; R', CH_2CH_3 JASMOLIN II
XI R, CH_3; R', H ALLETHRIN

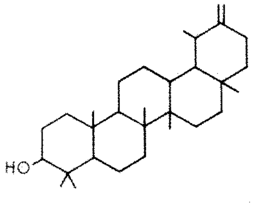


VII R", H CHRYSANTHEMUMIC ACID

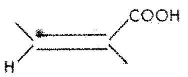
VIII R", CH_2CH_3 CHRYSANTHEMUMIC ACID ETHYL ESTER

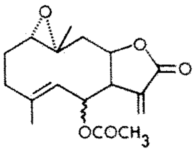


X CHRYSANTHEMIN (CYANIDIN CHLORIDE)



IX TARAXASTEROL (PYRETHROL)





XIII TIGLIC ACID

XII PYRETHROSIN

FIG. 1.—Chemical compounds, derived from pyrethrum, which were used for patch-testing.

METHODS AND MATERIALS

Dried pyrethrum flowers, extracts of the plants and samples of " Pyrethrins I ", which is a mixture of pyrethrin I (Fig. 1, I), cinerin I (II) and jasmolin I (III) and " Pyrethrins II ", which is a mixture of pyrethrin II (IV), cinerin II (V) and jasmolin II (VI), and also taraxasterol (pyrethrol, IX), were donated by Dr S. W. Head, Pyrethrum Marketing Board, Nakuru, Kenya.

Chrysanthemum monocarboxylic acid (chrysanthemumic acid, VII), chrysanthemumic acid ethyl ester (VIII), chrysanthemin (cyanidin chloride, X), and allethrin (XI) were obtained from Fluka AG Chemische Fabrik CH-9470, Buchs, Switzerland. Allethrin is an ester (dl-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one) of chrysanthemumic acid and, as prepared commercially, is a mixture of 8 optically active isomers. A sample of pyrethrosin (XII) was donated by Werner Herz, Ph.D., Department of Chemistry, Florida State University, Tallahassee 32306. Tiglic acid (XIII) was obtained from Aldrich Chemicals Co., 2371 North 30th Street, Milwaukee, Wis. Pyrethrum powder 10% and turpentine 2% in petrolatum were obtained from Hollister-Steir Laboratories, P.O. Box 3030, Calgary, Alberta. Neopentylglycol succinate and chromosorb W were obtained from Varian Associates, 1111 West Hastings, Vancouver.

*Separation of the Constituents of " Pyrethrins I and II "**

" Pyrethrins I " were separated from " Pyrethrins II " by ascending thin-layer chromatography on silica gel G using 1-hexane-ethyl acetate (3 : 1) as the developing solvent mixture. In this system, " Pyrethrins I " are well separated from " Pyrethrins II " (Stahl and Pfeifle, 1966). The latter were eluted from the support with ethyl ether and rechromatographed in a Brenner–Niederwisser chamber, using the solvent mixture 1-hexane-1-heptane-ethyl acetate (12 : 10 : 3). Repeated chromatography afforded pure pyrethrin II, cinerin II and jasmolin II. The purity of these materials was assessed by gas liquid chromatography after the method of Head (1966). The conditions were as follows: 1% neopentylglycol succinate on acid-washed chromosorb W (60–80 mesh), isothermal at 180°C and temperature programming at 4°C/min until a final temperature of 220°C was reached. The injector temperature used was 210°C and the helium flow was 60 ml/min.

Under these conditions cinerin I emerged at 3 min, jasmolin I at 4 min and pyrethrin I at 5·4 min. The constituents of " Pyrethrins II " were eluted only under temperature programming. Cinerin II emerged after 6 min of programming, jasmolin II after 7·6 min and pyrethrin II after 10·4 min.

A patient previously reported to be allergic to pyrethrum was patch tested to the compounds listed in Table I using A1-Test test patches obtained from IMECO Astra Ab, Stockholm, Sweden. The patient was numbered 1 in a previous report which related his case history (Mitchell *et al.*, 1970). Forty-eight hour closed patch tests were carried out on the skin of the upper back and scored by the method of Wilkinson *et al.* (1970). The compounds were without primary irritant effect for 3 adult white males in the concentrations used for testing.

RESULTS

The results of patch testing are shown in Table I. The patient showed positive responses only to pyrethrum flower heads and powder, to extracts of the plant, pyrethrosin and pyrethrin II. Pyrethrosin possesses an α-methylene group exocyclic to the γ-lactone (Barton and de Mayo, 1957) which we have reported

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* " Pyrethrins I " refers to the mixture of pyrethrin I, cinerin I and jasmolin I. " Pyrethrins II " refers to the mixture of pyrethrin II, cinerin II and jasmolin II.

TABLE I.—*Patch Test Reactions to Preparations of Pyrethrum and to Some Chemical Compounds Derived from Pyrethrum*

Compound	Vehicle	Concentration	Patch test results
Dried pyrethrum flower heads	—	As is	++
Pyrethrum powder	Petrolatum	2% w/w	++
Pyrethrum " marc " (extracted ground flowers)	Petrolatum	2% w/w	+++
Pyrethrum oleoresin (crude extract containing 30% pyrethrins)	Petrolatum	2% w/w	++
Fully dewaxed pyrethrum extract	Petrolatum	2% w/w	++
Refined pyrethrum extract (as used in aerosols)	Petrolatum	2% w/w	++
Mixture of Pyrethrin I, Cinerin I and Jasmolin I (Fig. 1, I–III)	98% aqueous ethanol	5% w/v	−
Mixture of Pyrethrin II, Cinerin II and Jasmolin II (Fig. 1, IV–VI)	98% aqueous ethanol	5% w/v	+
Cinerin II (V)	98% aqueous ethanol	2% w/v	−
Jasmolin II (VI)	98% aqueous ethanol	2% w/v	−
Pyrethrin II (IV)	98% aqueous ethanol	2% w/v	+
Allethrin (XI)	98% aqueous ethanol	2% w/v	−
Chrysanthemumic acid (VII)	80% aqueous ethanol	1% w/v	−
Chrysanthemumic acid ethyl ester (VIII)	80% aqueous ethanol	1% w/v	−
Chrysanthemin (X)	98% aqueous ethanol	1% w/v	−
Taraxasterol (IX)	Acetone	5% w/v	−
Tiglic acid (XIII)	80% aqueous ethanol	1% w/v	−
Pyrethrosin (XII)	80% aqueous ethanol	0·1% w/v	++
Pyrethrosin (XII)	80% aqueous ethanol	1·0% w/v	+++

20 μl of each of compounds I–XIII was applied to the patch and allowed to dry before application of the patch to the skin.

elsewhere to be the immunologically active group of these compounds (Mitchell *et al.*, 1970; Mitchell and Dupuis, 1971).

Pyrethrum " marc " (extracted ground flowers) produced the strongest patch test reaction, followed by pyrethrum oleoresin fractions (prepared by distillation procedures), pyrethrosin, Fig. 1 (XII), and pyrethrin II, Fig. I (IV).

Pyrethrosin produced ++ positive reactions in a concentration 0·1% w/v; pyrethrin II produced + positive reactions in a concentration 2% w/v.

Pyrethrin I and the 6 other esters of chrysanthemumic acid which were tested produced negative patch test results.

DISCUSSION

Acetic acid is present in dried pyrethrum flowers and may produce primary irritation of the skin (Frank and McGeachin, 1949). The ether-soluble fraction of the alcohol extractive of pyrethrum " marc " (extracted ground flowers) contains a phenolic constituent, chrycinnerol, which possesses primary irritant properties, but pyrethrins themselves are not primary irritants (Subbaratnam and Siddiqui, 1956).

Previous workers reported observations which suggested that pyrethrin did not represent the allergenic principle of pyrethrum powder. Thus, Martin and Hester (1941) noted that pyrethrins were known to be concentrated in the ovaries, whereas the receptacles and the involucres of the plants contained only small amounts of the insecticidal principles. However, patch tests showed that the allergenic principles were present in all the parts of the flower head which were tested. In the case of one allergic individual, a concentrate of pyrethrins (93%) produced only a very slight reaction, whereas a dried flower head applied, as is, produced a strong positive reaction.

Moreover, pyrethrum extracts remained allergenic after extraction with petroleum ether which removed the pyrethrins. They observed that the allergen was thermostable at 100°C and present in the volatile oil of pyrethrum extract.

Pyrethrosin possesses the above chemical characteristics.

One of the patients of Martin and Hester (1941) developed dermatitis after approaching a field of flowers in bloom and also after opening a can of dried flowers indoors. Since patch tests to pollen produced only a slight positive reaction, the authors concluded that a volatile oil rather than the pollen was responsible for such dermatitis.

Pyrethrin II has a chemical formula close to that of pyrethrin I and quite unlike that of the sesquiterpene lactone, pyrethrosin; all of the 3 compounds named possess an unsaturated methylene group.

It seems that pyrethrins, the insecticidal esters of chrysanthemumic acid, are not the principal allergenic fractions of pyrethrum, whereas pyrethrosin, which does not have insecticidal properties, has the immunochemical requisite of this group of lactones and is probably the principal allergenic fraction of pyrethrum.

Respiratory allergy caused by pyrethrum powder was not due to the presence of pyrethrins (Garratt and Bigger, 1923; Feinberg 1934; Zucker, 1965).

It has occasionally been reported that patients sensitive to pyrethrum and ragweed (*Ambrosia*) may also react to turpentine. The patients whom we have found sensitive to sesquiterpene lactones derived from pyrethrum and ragweeds have shown negative patch test reactions to turpentine.

Turpentines from different countries may contain Δ_3-carene, α-pinene, β-pinene, camphene, myrcene and limonene. Δ_3-carene or its oxidation products (peroxides) appear to be the principal sensitizers present in turpentines. We known of no reports of the presence of sesquiterpene lactones in turpentine or its degradation products, and such cross-sensitivity, if indeed it exists, requires further precise study of the constituents of the various turpentines (Editorial, *Trans. a. Rep. St John's Hosp. derm. Soc. Lond.*, 1971).

Concerning the industrial importance of our observations, about twenty thousand tons of pyrethrum are produced annually for insecticidal purposes. but it is highly unlikely, if not impossible, that pyrethrosin should be present in commercial refined pyrethrum extracts (Head, 1970). Moreover, such extracts for agricultural or household use contain smaller quantities of pyrethrin than we have used for patch testing (e.g. in one commercial aerosol-type dog flea spray, which may be purchased at a local pet shop for household use, pyrethrins are present in a concentration of 0·06%). Nevertheless, pyrethrum sensitization of the skin is of some practical importance. For example, Baer (1971) found positive patch tests to pyrethrum in 3·1% of 540 patients who were examined at an eczema clinic in New York, N.Y.

As DDT falls into disuse for disinfestation of household pets, pyrethrum may become more widely used for this purpose in the future; and, if this should be so, the opportunities for exposure and for sensitization to pyrethrum will increase.

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ADDENDUM

We are informed by Dr S. W. Head (personal communication, 1972) that the concentration of pyrethins II in the non-volatile portion of an aerosol spray would be at the most 0·15%; however, after evaporation of the propellant, the concentration of pyrethrins II will be between 3 and 5 times greater in the case of an oil based aerosol i.e. a maximum of 0·75%.

Br. J. Derm. (1972) **86**, 574.

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EMOTIONS AND THE SKIN: THE CONDITIONING OF SCRATCH RESPONSES IN CASES OF ATOPIC DERMATITIS

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Summary.—To test the hypothesis that patients with atopic dermatitis would react with a scratch response to a variety of environmental signals a group of 18 patients and a group of normal controls, matched for age and sex, took part in a conditioning experiment with an itch as the unconditioned stimulus (U.C.S.). The dermatological patients were found to differ from the controls in terms of several personality measures, notably anxiety and hostility, and in the number of galvanic skin responses and scratch responses they produced during the conditioning sessions. The implications of these findings are explored and a tentative hypothesis about the aetiology and nature of atopic dermatitis is proposed.

THE belief that emotional disturbance is mirrored by changes in the skin is neither new nor contestable. Everyday language confirms the opinion, but tells us little about the mechanisms whereby physiological and even pathological alteration of skin structure and function are caused by the postulated or observed psychological disturbance. As Witts (1948) has observed; " How easy it is to aver that mental states or ideas can be converted into physical disease processes . . . but how difficult it is to prove it or to demonstrate the mechanism ".

The great majority of investigators, after selecting a particular dermatosis, have proceeded to study, mainly by clinical methods, the personality structures of their subjects. Only in comparatively recent times have more precise psychometric techniques been used for this purpose. That a particular constellation of traits and characteristics, conscious and unconscious conflicts and presumed difficulties in inter-personal relationships, in early or later life, are commonly observed in patients with a variety of dermatoses has led a number of investigators to assume too readily that these psychological experiences are aetiologically related to the skin disorders. There are formidable objections to accepting this opinion. Many of the studies have been inadequately controlled; the psychological conflicts uncovered by psychoanalytical explorations and projective testing appear not to be specific for a particular dermatosis; some at least of the psychological disturbances may be the consequences rather than the causes of the illness; often there is a lack of diagnostic homogeneity of cases discussed together under the general heading of, for example, " neuro-dermatitis "; and few investigators have been able to demonstrate an agreed psychosomatic mechanism mediating between personality disorder and chronic relapsing and remitting dermatoses. These and other difficulties have led to a virtual disappearance of this method of psychosomatic investigation. Many of the earlier studies of this kind have been summarized by Obermayer (1955) and more recent ones by Brown (1967).

A more valuable contribution has come from measurements of physiological change in those skin disorders in whose aetiology emotional disturbance is thought to play an important part. Graham and Wolf (1953) demonstrated an increase

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in itching, scratching and skin temperature in eczema patients with whom emotionally disturbing topics were being discussed. The investigations of Mason and Black (1958), Black (1963a, b) and Black and Friedman (1965) have shown that histological and other changes in the skin associated with allergic responses can be influenced by hypnosis, while treatment by hypnosis of actual dermatoses—organic or presumably psychogenic—has led sometimes to startling improvement (Mason, 1952; Asher, 1956; Bethune and Kidd, 1961). Other writers (Moody, 1946; Ullman, 1947) have under hypnosis produced skin lesions such as blisters and weals. The relationship of emotional state to autoerythrocyte sensitization in patients with unusual purpuric lesions of the skin, including stigmata, has been described by Agle and Ratnoff (1962). Kepecs *et al.* (1951) used an ingenious technique to demonstrate increased serum exudation in the skin that could then be related to emotional disturbances, while Grant *et al.* (1936) and Hopkins *et al.* (1938) have taken note particularly of cholinergic mechanisms underlying some varieties of emotionally produced urticaria.

Perhaps of all the dermatoses that have been studied intensively by psychosomatic techniques, atopic dermatitis must take pride of place. Atopy is defined by Rook *et al.* (1968) as " a genetically determined disorder in which there is an increased liability for reagin antibodies and increased susceptibility to certain diseases, especially asthma, hay fever and atopic dermatitis ". These authors claim that an hereditary basis to atopic dermatitis can be demonstrated in 70% of cases, and conclude that the sufferer has a basically irritable skin that responds by itching when stimulated in a variety of ways. Psychological factors play a variable and often dominant role in the precipitation and perpetuation of the lesions in some cases. Cairns (1968) has observed that itch thresholds can be lowered by psychological factors and that " the repetition of previous experiences . . . may engender fear with paroxysms of rubbing ". Many writers have recorded spontaneous scratching on the part of patients with atopic dermatitis when emotionally disturbing subjects are under discussion; but whether this is a response to an itch sensation caused by some central mechanism (Whitlock, 1957) or to peripheral changes is not wholly clear. The unusual vascular response to local stimulation in patients with atopic dermatitis is well known (Rothman, 1954), but the delayed blanch reaction to cholinergic drugs may not be due to vaso-constriction but to increased exudation of fluid following vaso-dilatation (Ramsay, 1969). Such a phenomenon might in its turn lead to lowered itch thresholds.

The cause of itching in atopic dermatitis seems not to be entirely understood. However, the reports by Arthur and Shelley (1957, 1958) and Rajka (1968) point to the release of proteolytic enzymes as one possible precipitant of itching. Injection of these substances into areas of skin affected by the disease leads to itching of greater intensity and duration than that experienced by controls. One might postulate that similar endogenous enzymes in atopic dermatitis patients can be released by a variety of stimuli, leading to local vaso-dilatation and intradermal exudation of fluid.

Reviews of the work on psychological characteristics of patients with atopic dermatitis (Obermayer, 1955; Brown, 1967) have concluded that most eczema patients had vulnerable personalities and displayed some form of emotional disturbance. However, the majority of writers have noted a variety of prominent personality traits in neuro-dermatitis sufferers, although running through this voluminous literature the themes of obsessionality and concealed hostility frequently recur. It has been postulated that such patients, when faced by frustrating situations, turn some of their unexpressable anger on themselves and so produce the characteristic lesions of atopic dermatitis. It should be noted that many normal persons tend to scratch in moments of frustration or embarrassment, and personal observation has recorded how often this scratching takes place at sites that are typically affected by atopic dermatitis. Nevertheless, before assuming too readily that personality traits of hostility, neuroticism and obsessionality are inevitable precursors of the disease, one must recall the work of Kidd and Watt (1967) and of Meyer *et al.* (1968), which demonstrated that in some cases the duration and location of the lesions can have strong influences on the results of psychological tests and the clinical manifestations of emotional disturbance. In any case, the majority of patients with atopic dermatitis develop their first lesions in early infancy at a time when the personality qualities observed in adult sufferers could hardly be operating. One could argue, therefore, that physical restraint and the general management of an itchy dermatosis in infancy could lead to feelings of frustration and rage that had to be concealed over long periods of childhood. The repeated injunction: " Stop scratching " must in the end have some effect on the child's behaviour and inner feelings.

It appeared to us from a study both of the dermatosis and the relevant literature that psychological factors played an important part in some cases of atopic dermatitis; and that their influence would vary throughout the patient's lifetime. Close observation of such patients has shown that they scratch readily and that such scratching can be provoked by emotionally disturbing topics. We postulated, therefore, that with these patients, because of past experience, scratch responses would occur in response to a variety of environmental signals. We also thought that these patients would have a more persistent response to an itch stimulus; i.e. that such patients would take longer to habituate as measured by the galvanic skin response to an itch sensation. Finally, we assumed that atopic dermatitis patients would develop more rapidly than controls a conditioned scratch response to signals linked with an itch stimulus and that, once established, such responses would extinguish more slowly than in controls.

In order to test these hypotheses a group of patients with atopic dermatitis was selected and an experiment was designed, based on the phenomena of the galvanic skin reflex (G.S.R.). This physiological measure was chosen for several reasons: (1) There is a large literature on studies of conditioning of this response; (2) we required a sensitive index of reactivity; (3) we needed to make continuous recordings during the experimental sessions. Although controversy exists over the appropriate unit of measurement of the G.S.R. (Darrow, 1964; Forbes, 1964), this debate is not relevant to the present study which restricts itself to qualitative, rather than quantitative, measurements of the G.S.R. in relation to specific stimuli. The main conditioning response used in this study is a behavioural one, a scratch response.

METHODS

The Patients

Eighteen patients, 6 male and 12 female, with a diagnosis of atopic dermatitis made by our dermatological colleagues, were examined. The majority had active lesions in the usual flexural sites, but some had more generalized eruptions on face and trunk.

The average age of the patients was 25·3 years, with an age range of 14 to 42 years. In 16 of these patients the dermatitis first appeared during infancy or early childhood. Fourteen of the patients (78%) had a definite history of asthma, hay fever, migraine or urticaria, and there was a positive family history of one or more of these conditions in 13 (72%) cases. Twelve patients felt that emotional factors could sometimes cause or exacerbate their skin condition. Many patients had also noted the ill effects of climate and contact with wool. Two of the female patients had noted an improvement of their skin during pregnancy, while 3 said they were definitely worse during this period. Clinical assessment of personality showed that 4 patients were essentially normal, while 3 showed sociopathic or histrionic traits, 7 had mild obsessional traits and 10 had passive-aggressive qualities. Feelings of anger and frustration that could not be expressed were experienced by this group of 10 patients.

The Controls

Eighteen patients under investigation in the medical wards and matched for age and sex with the skin patients were used as controls. Their average age was 25·4 years, and the age range was 13 to 41 years. Five of these patients were recovering from infectious hepatitis, 5 had neurological disorders, 3 were suffering from hypertension or chronic heart disease, 2 were being investigated for hyperthyroidism and 3 other patients had a variety of symptoms of uncertain aetiology. Five patients (28%) gave a past history of urticaria, hay fever or migraine; but in only one case was there a positive family history of allergic disorder (urticaria). Eleven of the control patients were judged by clinical examination to show normal personality qualities, 3 had mild obsessional traits, 2 were classed as sociopathic or histrionic and 2 others had evidence of emotional instability and anxiety.

Psychological Investigations

Psychometric data were obtained by administration of the Cattell Personality Inventory (16 P.F., Cattell and Eber, 1957), the Eysenck Personality Inventory (E.P.I., Eysenck and Eysenck, 1964), the Buss–Durkee Hostility Inventory (1957) and Welsh's Additional M.M.P.I. (Minnesota Multiphasic Personality Inventory) scales of anxiety and repression (Welsh and Dahlstrom, 1956). In addition, comprehensive social and psychological histories were taken from both patients and controls.

Experimental Techniques

The conditioning experiments were conducted in a sound-reduced, temperature controlled laboratory (20° ± 1°C) and consisted of 5 parts.

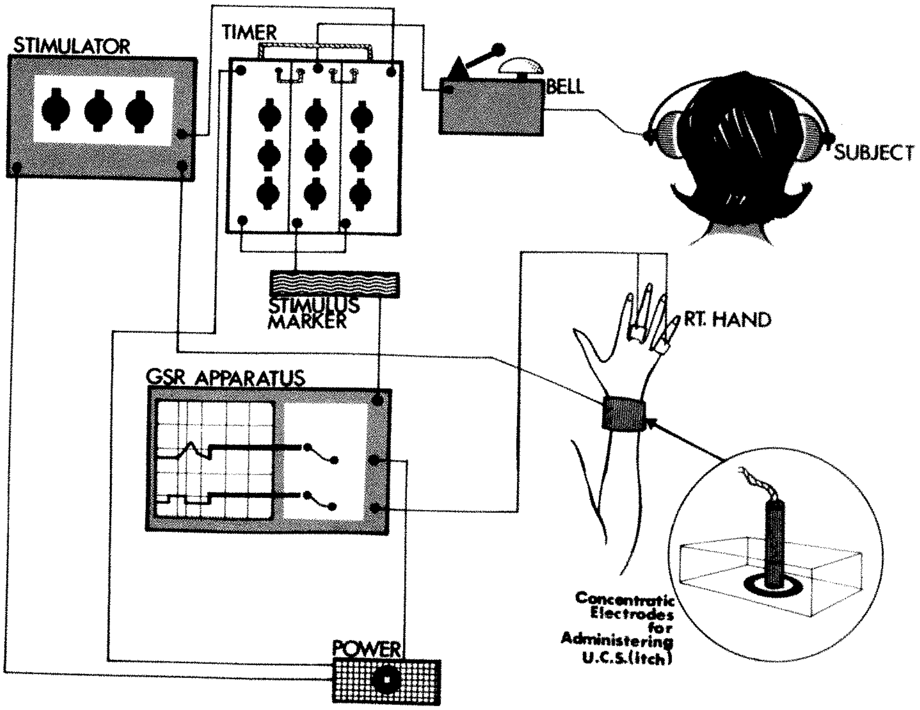
1. Determination of patient's threshold to an itch stimulus which was used as the unconditional stimulus (U.C.S.).
2. Presentation of the U.C.S. to test habituation.
3. Presentation of the conditional stimulus (C.S.), a tone, in order to allow habituation to take place.
4. The actual conditioning phase of the experiment.
5. The extinction phase.

Apparatus (see diagram)

The itch stimulus (U.C.S.) was produced by application to the skin of 2 concentric electrodes, one made of brass and the other of a brass-coated tin base alloy set into a perspex holder. This instrument was held firmly in contact with the skin by use of a crepe bandage. The electrodes were connected to a Grass Square Wave Stimulator, which in its turn was connected to a stimulus marker on the G.S.R. recording apparatus. At threshold stimulus a pricking, itching sensation could be perceived at the site of the electrodes. This could become painful if the strength of the stimulus was increased excessively.

The G.S.R. electrodes, consisting of silver plated fittings with a velcro strap, were connected to a Stoelting Dermograph which recorded skin resistance.

The conditional stimulus (C.S.) was produced by a modified bell of constant loudness (approximately 60 decibels), and this was connected to a different stimulus marker on the dermograph. The instruments responsible for the C.S. and the U.C.S. were connected to the first 2 modules of the automatic timer, a third module being used to vary the time interval between presentations of the paired C.S. and U.C.S. stimuli.



The equipment was placed outside the laboratory and all observations of the patients were made by means of a one-way mirror, with instructions being given during the experiments through a 2-way intercommunication system. The patient was made aomfortable in the lying position and the details of the experiment were explained, clthough no mention was made of the conditioning procedure. We stated that the aim of the experiment was to measure the patient's threshold for an itch stimulus and to compare his skin reaction (by means of the G.S.R.) to this itch with that caused by a " neutral " stimulus (the tone). The U.C.S. (itch) stimulator was applied to the anterior aspect of the right wrist medial to the tendon of the flexor carpi radialis muscle and proximal to the first skin crease after the area had been cleaned with ethyl alcohol. The G.S.R. electrodes were applied to the palmar aspects of the thumb and forefinger of the right hand, the area under the electrodes having first been coated with Beck-Lee E.E.G. paste. Earphones were placed on the patient's head and instructions to relax and to keep the right arm as still as possible during the experiment were repeated. The experimenter then left the room.

The Experiments

Part 1—*Determination of itch threshold.*—The patient was instructed to report the moment at which he first felt the itch stimulus on his wrist. The stimulus—starting point 1·5 V.—was applied for 5 seconds at 20-second intervals and increased by 0·5 V. at each presentation until the patient felt an itch sensation. The intensity of the stimulus was varied about this point until a reasonably good estimate of the patient's threshold could be determined. This threshold value was recorded.

Part 2—*Habituation.*—The intensity of the itch stimulus was increased by 1 V. in order to increase it a little above threshold level, and the patient was told that the stimulus would be presented a few times in order to test his skin reaction to it, his only task being to remain as relaxed and as still as possible. After switching on the G.S.R. recorder no stimulation was presented for 3 min or until the patient's record ceased to show major spontaneous fluctuations. The itch stimulus (U.C.S.) was then presented 10 times at random intervals of 13 or 23 seconds between presentations. Each stimulus was of 5 seconds duration. The presence or absence of a specific G.S.R. to the stimulus was noted on each occasion.

Part 3—*Habituation of the C.S.* (*tone*).—As an orienting response is produced on the presentation of any stimulus, the conditional stimulus had to be presented a number of times to allow habituation to take place and to ensure that the responses at the beginning of the conditioning phase would not be made up largely of orienting responses to the novel stimulus (C.S.).

The patient was given the same instructions as previously, viz. to relax and remain as still as possible during the recording. Once again the G.S.R. recorder was switched on and 3 min were allowed for adaptation before the first presentation of the C.S. Ten presentations of the tone (each of 3 seconds' duration) were given, with a random interval of 15 or 25 seconds between presentations, and the presence or absence of a specific G.S.R. response to each presentation of the stimulus was noted.

Part 4—*The conditioning phase.*—Before starting the conditioning experiments, the patient's threshold for the perception of the itch stimulus was redetermined. In cases where this had altered in comparison with the original findings, the new values (+ 1 V.) were used, thus ensuring that the patient was still perceiving the itch stimulus during this phase of the experiment.

The patient was now instructed to scratch lightly on the bandage retaining the electrodes responsible for the itch stimulus whenever he perceived the itch (U.C.S.) following upon termination of the tone (C.S.). Forty C.S.–U.C.S. trials were presented with 75% intermittent reinforcement, i.e. a randomized 10 trials consisted of the tone (C.S.) unaccompanied by the itch stimulus. The duration of the C.S. was 3 seconds, the C.S.–U.C.S. interval was 0·1 seconds, the duration of the U.C.S. was 5 seconds and the inter-trial interval was randomized at 10 or 20 seconds. Each scratch response and G.S.R. associated with the C.S.–U.C.S. (or C.S. alone) presentation was noted.

Part 5—*Extinction phase.*—This followed the conditioning phase without a pause and consisted of 10 presentations of the C.S. alone, each of 3 seconds' duration and with an interval of 15 or 25 seconds (randomized) between 2 presentations. Any scratch responses made or G.S.R.s recorded following presentations of the stimulus during this period were again noted. Finally, the patient's itch threshold was again checked, using the same method as outlined previously, in order to see if any alterations in this threshold had taken place since the last estimate.

RESULTS

Results of the psychometric tests and psychological experiments for both patient and control groups are set out in Tables I, II. Significances were tested by the sign test, since assumptions about normality of distribution of the data could not be made.

Table I shows that there are significant differences between the patient and control groups on several scales of the tests administered. The only difference in the 16 P.F. scales between patients and controls lies in the Q4 scale—a measure of internal tension in the form of irritability, anxiety and worry—where the patients exhibit a significantly higher score than the controls. None of the second order factors of the 16 P.F. (neuroticism, anxiety or introversion) show significant differences between the 2 groups.

The neuroticism score of the E.P.I. shows the patient group to be significantly more neurotic ($P = < 0{\cdot}004$) than the control group, whereas there is no difference between the 2 groups in terms of extraversion. The M.M.P.I. Additional Anxiety Scale shows that the patient group is significantly more anxious than the control group ($P = < 0{\cdot}011$), while the Welsh R factor (denial) shows no significant difference.

TABLE I

Psychometric tests		Means		
		Patient group	Control group	*P*
16 P.F.	Scale A	5·5	5·4	N.S.
	B	6·5	6·5	N.S.
	C	5·1	5·8	N.S.
	E	5·0	5·9	N.S.
	F	5·2	5·7	N.S.
	G	5·9	5·4	N.S.
	H	4·8	5·6	N.S.
	I	4·9	5·4	N.S.
	L	5·9	5·1	N.S.
	M	5·9	5·3	N.S.
	N	5·9	5·6	N.S.
	O	6·9	5·7	N.S.
	Q1	5·7	6·1	N.S.
	Q2	6·5	5·9	N.S.
	Q3	5·6	5·5	N.S.
	Q4	7·3	5·0	<0·006
	2nd order factors			
	Neuroticism	6·5	4·9	N.S.
	Anxiety	6·7	5·2	N.S.
	Introversion	4·6	5·6	N.S.
E.P.I.	Neuroticism	15·4	9·0	<0·004
	Extraversion	13·2	13·4	N.S.
M.M.P.I. (Welsh) additional	Anxiety	19·8	9·8	<0·011
	Denial	17·9	19·1	N.S.
Buss–Durkee hostility	Assault	4·3	4·1	N.S.
	Indirect	4·3	4·4	N.S.
	Irritability	5·7	4·0	<0·038
	Negativism	2·0	2·9	N.S.
	Resentment	4·1	1·4	<0·018
	Suspicion	3·1	3·1	N.S.
	Verbal	3·9	4·0	N.S.
	Guilt	5·9	4·0	<0·018
	Factor I (hostility)	7·2	4·5	<0·048
	Factor II (aggression)	18·2	16·5	N.S.
	Total score (hostility–aggression)	25·4	21·0	<0·015

The Buss–Durkee Hostility Scale shows that the patient population differs on 3 dimensions from the controls, while 5 dimensions show no significant differences between the 2 groups. The 3 scales on which the patients exhibited a significantly higher score than the controls were irritability, resentment and guilt. The patients were also found to have a higher Factor 1 score, which is a composite figure derived from the sum of scores on the resentment and suspicion scales, both indicative of unexpressed hostility. On the other hand, scores for Factor 2, that is, expressed hostility, obtained by adding together the scores on the assault, indirect irritability and verbal scales, were not significantly different. The total hostility–aggression—the sum of scores on Factors 1 and 2—was significantly higher for the patients than for the controls ($P = < 0{\cdot}015$).

Table II shows the mean scores for the number of trials taken to habituate both the U.C.S. (itch) and the C.S. (tone) and the number of conditioned responses during both the conditioning and extinction phases of Parts 4 and 5 of the experiments. Significant differences were found between the number of trials required to habituate the C.S. (tone) and the number of conditioned scratch responses and G.S.R.s during the conditioning phase. The number of trials needed to habituate the U.C.S. (itch) and the number of conditioned responses during the extinction phase showed no significant differences between the patient and the control groups. It is to be noted, however, that the differences, although not statistically significant, were in the expected direction.

TABLE II

Parameter	Means		
	Patient group	Control group	*P*
No. of trials to habituate* U.C.S. (itch)	5·3	4·2	N.S.
No. of trials to habituate* C.S. (tone)	8·0	6·0	<0·002
Conditioning phase			
No. of conditioned scratch responses	2·1	0·05	<0·035
No. of conditioned G.S.R.s	3·8	0·6	<0·029
Extinction phase			
No. of conditioned scratch responses	1·12	0·5	N.S.
No. of conditioned G.S.R.s	2·43	1·06	N.S.

* The criterion for habituation of both the U.C.S. and the C.S. followed that chosen by Lader and Wing (1964), who took 3 presentations of the stimulus without a response to mean that habituation had taken place (any further responses being credited to dishabituation).

DISCUSSION

Despite the variety of psychometric scales used, the only dimensions that differentiated the skin population from the control group related to hostility, anxiety and neuroticism. Another interesting finding arose from the use of the Buss–Durkee Hostility Inventory, which divides the total score obtained on the Inventory into 2 factors, the first relating to felt hostility and the second to manifest aggression. This study shows that the skin patient group expressed their aggression as frequently as the control group, although some previous studies (Stokes, 1930; Graham and Wolf, 1953; Rush *et al.*, 1957) have implied that manifest aggressive behaviour was more controlled in atopic dermatitis patients. On the other hand, our patients' total hostility score was higher, suggesting that latent hostility—especially in terms of resentment—was significantly greater than that manifested by the control group.

Our failure to demonstrate that the skin patients would take longer to habitu to the itch stimulus may be the consequence of 2 facts: the comparatively sn number of subjects and our recording of the qualitative aspects of the G.S alone. Our findings on this point, although in the expected direction, did achieve statistical significance, and one can but speculate whether larger numl of subjects would have produced a significant result. On examining the G.S. of both patient and control groups for *quantitative* differences during the U.(habituation phase, the responses from the patient group appeared to be much lar than those of the control group, even although there were not significantly mor them. This suggests that sensitivity in terms of amplitude of the G.S.Rs rat than the number of trials needed to habituate the orienting response should h been examined. In fact, the significant difference between the experimental control groups for the number of trials to habituate the C.S. (tone) could be to the finding that more of the skin patients than the controls showed augmen G.S.R.s when the number of presentations of the C.S. was increased. T implied that, in spite of the relatively " neutral " nature of the stimulus, more the skin patients reacted with a defensive reflex to its presentation. This defens reflex is not subject to habituation in the same way as the orienting reflex therefore produces higher scores for the number of trials to habituate the C.S.

The possibility that the habituation phenomenon may be associated w autonomic reactivity (Eysenck, 1967) was considered, but correlations of neuroticism scores of the E.P.I. (which according to Eysenck measures autono reactivity) with (a) the number of trials to habituate the U.C.S. (itch), and (b) number of trials to habituate the C.S. (tone) produced insignificant resu ($r = + 0{\cdot}012$ and $r = - 0{\cdot}088$ respectively).

Since Franks (1956) demonstrated that conditionability was related to extraversion–introversion dimension of the E.P.I. and Spence and Taylor (19 showed conditionability to be related to anxiety as measured by Taylor's Mani Anxiety Scale, rank order correlations were carried out between the extravers score of the E.P.I., the Welsh Anxiety Score and the number of conditioned scra responses and conditioned G.S.R.s. As the M.A.S. score used by Spence Taylor correlates extremely highly with the neuroticism score of the E.P correlations were also carried out between the *N* score of the E.P.I. and the num of conditioned responses. Results are shown in Table III and, although neit the neuroticism nor the extraversion scores correlate with conditionability, Welsh Anxiety score correlates with conditionability at the 5% level of significan

TABLE III.—*Correlations Between Number of Conditioned Responses and Neuroticism, Extraversion and Anxiety Scores (Welsh)* (*d.f.* = 34)

	No. of conditioned scratch responses			No. of conditioned G.S.R.s		
	r	*t*	*P*	*r*	*t*	*P*
N score	0·209	1·25	N.S.	0·233	1·39	N.S.
E score	0·109	0·64	N.S.	0·328	2·02	N.S.
A score (Welsh)	0·348	2·16	0·05	0·371	2·33	0·05

To test whether the difference between the skin patients and the control gro in ease of forming a conditioned response to an itch stimulus was due solely to increased anxiety score for the experimental group, rank order correlations w carried out between the anxiety scores and the number of conditioned responses (scratch responses and G.S.R.s) for both the experimental and the control groups separately. All correlations were non-significant (see Table IV). This analysis suggests that there is a real difference between the ability of the psychosomatic skin patients in this study and the control group to form a conditioned response to an itch stimulus that is beyond that attributable to the effects of anxiety.

TABLE IV.—*Correlations Between Number of Conditioned Responses and Anxiety Score (Welsh) for Patients and Controls* ($d.f. = 16$)

	Anxiety score and number of conditioned scratch responses			Anxiety score and number of conditioned G.S.R.s		
Group	r	t	P	r	t	P
Experimental	0·114	0·46	N.S.	0·068	0·27	N.S.
Control	0·399	1·71	N.S.	0·391	1·70	N.S.

Consideration of our results makes it possible to propose a tentative hypothesis about the aetiology and nature of atopic dermatitis. As with most psychosomatic disorders, in atopic dermatitis there is a strong hereditary component that appears to be responsible for a basically irritable skin. A variety of exogenous and endogenous stimuli—singly or in combination—will lead to vaso-motor changes that either cause localized itching or lower the itch threshold of the skin. In either case the result is scratching on the part of the patient, particularly at the sites that are both easily accessible and particularly prone to be scratched when a patient experiences emotional tension. From what we know of the psychological make-up of these patients and the course of their illnesses from their first appearance in early childhood, one might predict that signals capable of arousing feelings of resentment and hostility would be particularly liable to cause local vaso-motor changes that in their turn would lead to itching and scratching. Alternatively, as our work suggests, such signals will precipitate a conditioned scratch response that in its turn will produce local vaso-motor changes and consequent aggravation of itching and scratching. Our experiments do not tell us whether the initial scratch response could be due to centrally caused itching that could arise from feelings of resentment and hostility originating from a variety of environmental stimuli.

However, whatever the theoretical implications might be, it does appear that, in some patients at least, techniques of deconditioning such as those developed by behaviour therapists might be worthy of a trial. Quite obviously, psychological treatment of this kind would be of value only in patients where a strong emotional contribution to aetiology can be demonstrated and might not be of great help to those in whom specific allergens can be shown to be responsible for relapses.

In any case, it should not be assumed that psychological treatment alone will " cure " these patients, although careful selection of patients showing manifest emotional disturbance will single out those who might derive most benefit from psychiatric help (Brown and Bettley, 1971).

Finally, it must be said that our techniques of study require refinement by the use of more sophisticated equipment, thus permitting quantification of G.S.R. data. In addition, we need to examine another control group, matched with respect to anxiety scores with the experimental population, to determine more precisely the respective roles of anxiety and conditionability in the production of the differences that we have recorded by comparatively crude methods. However, having established a particular technique, we hope that this type of study will be repeated with larger numbers of patients suffering not only from atopic dermatitis but also from chronic or recurrent pruritic eruptions such as urticaria, lichen simplex and ano-genital pruritis.

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CHALONE CONTROL OF MITOTIC ACTIVITY IN ECCRINE SWEAT GLANDS

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Summary.—In the eccrine glands of mouse footpads mitoses are common in the intradermal ducts, and they also occur in the ampullae. No mitoses were seen in either the gland cells or the intraepidermal ducts.

The duct cell mitoses were not inhibited by either the epidermal or the sebaceous gland chalones. However, it was found that human sweat contains an inhibitor of eccrine duct mitosis, which does not inhibit either epidermal or sebaceous gland mitosis. This sweat inhibitor has the characteristics of an eccrine gland chalone. In support of this it was found that adrenalin inhibits and adrenalectomy promotes eccrine duct mitosis.

In recent years it has been shown that mitotic activity in many of the tissues of the skin is controlled by specific chalones, each of which is synthesized within the same tissue on which it selectively acts as a mitotic inhibitor (Bullough, 1971; Bullough and Deol, 1971). Thus there is an epidermal chalone (Bullough and Laurence, 1964a, b), a sebaceous gland chalone (Bullough and Laurence, 1970), and a melanocyte chalone (Bullough and Laurence, 1968a), and it is also clear that specific mitotic control mechanisms exist in the hair follicle (Bullough, 1967) and in the various dermal tissues (Bullough and Deol, 1971).

Little attention has so far been paid to the mitotic activity of the sweat glands. In human eccrine glands some mitotic activity has been reported in the ducts, but little has been seen in the glands themselves (Lobitz *et al.*, 1954; Christophers and Braun-Falco, 1967). There is even less information regarding apocrine glands; but, in the pig, Winter (1971) has found mitoses in the duct cells.

In the present study of the eccrine sweat glands of mouse footpads the mitotic regions are defined and the mitotic control mechanism is considered.

MATERIALS AND METHODS

The Mice

All the animals used were adult male C57 black mice between 4 and 6 months old. Four hours before killing, each was injected with 0·1 mg colcemid in 0·25 ml water to arrest the developing mitoses in the metaphase. All injections were given at 11.00 hours, which was the beginning of a sleep period.

The feet were fixed whole in Bouin's alcoholic fluid and stored in 70% alcohol. The soles of the right hind feet were then carefully dissected from the ligaments and bones, and were sectioned at 7 μm. Only the large glands massed within the swollen foot pads were studied.

Measurement of Mitotic Activity

The mitotic activity was studied in the walls of the intradermal ducts which, since they twist and turn within the dermis, were cut at all angles from the longitudinal to

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the transverse. To meet this difficulty the mitotic counts were made by the method previously developed for sebaceous glands (Bullough and Laurence, 1970). The total number of mitoses was counted in 25 successive duct sections, which were of widely differing sizes according to the angle at which they were cut. For each mouse foot this procedure was repeated 5 times, and the average figure so obtained was taken as representative of that mouse. Each experimental group contained from 5 to 60 mice and, from the individual averages, a grand average and standard error were calculated.

Tissue Extracts and Hormones

Except for those that were adrenalectomized, all the mice in the experimental groups received a single injection of a tissue extract or of a hormone at the same time as they were injected with colcemid. The tissue extracts used were: a 71–80% ethanol precipitate of a water extract of pig skin, which was known to contain both the epidermal and the sebaceous gland chalones (Bullough *et al.*, 1964; Hondius Boldingh and Laurence, 1968; Bullough and Laurence, 1970); a 71–80% ethanol precipitate of a water extract of pig palate, which was known to contain the epidermal chalone without the sebaceous gland chalone (Bullough and Laurence, 1970); and freshly collected human sweat. The pig skin and pig palate extracts were injected subcutaneously in doses that were known to be adequate to cause a significant mitotic depression in the epidermis and sebaceous glands. The human sweat was injected undiluted.

The hormones used were adrenalin bitartrate and hydrocortisone. In calculating the dose of adrenalin, the weight of the bitartrate was excluded.

OBSERVATIONS

Gland Structure and Mitotic Activity

Eccrine-type sweat glands are present on the ventral surfaces of all the digits, but on the soles of the feet they are restricted to the foot pads. Those in the foot pads are larger and more crowded than those on the digits, and indeed the large size of the pads is due mainly to the mass of eccrine glands within them.

Each gland consists of 4 main regions: (i) the tightly coiled glandular tubule opens into (ii) a short "ampulla", of doubtful function (Loewenthal, 1961), which leads into (iii) the relatively straight intradermal duct, which in turn leads into (iv) the coiled intraepidermal duct.

Not a single mitosis was seen among the gland cells in any of the thousands of sections examined, and mitotic activity seems to be restricted to the ampulla cells and the intradermal duct cells. The ampulla is commonly folded among the coiled tubules of the gland. It is distinguished from the intradermal duct by the fact that its cells form a single and not a double layered epithelium, by its smaller and more closely crowded nuclei, and by its larger lumen. The fate of the new cells formed by mitosis in the ampulla epithelium is not obvious.

The intradermal duct is lined by 2 cell layers: mitotic activity is confined to the outer cell layer, which is based on a connective tissue sheath; the inner cells, which line the duct lumen, are post-mitotic. The outer cells appear to be firmly anchored to the connective tissue and are therefore immobile. The newly formed post-mitotic cells continuously join the inner lumenal cells, and the pressure so caused forces the lumenal cells to slide outwards, evidently as an unbroken tube, to be lost at the epidermal surface. The intraepidermal duct is composed of only a single layer of these outward-moving, post-mitotic, lumenal cells.

Where the intradermal duct leaves the dermis there is a cone-shaped, epidermal depression. The basal cells of this cone are indistinguishable from basal epidermal cells and are actively mitotic. The more distal cone cells form a "periductal sheath" (Lobitz *et al.*, 1954) of prickle cells around the intraepidermal eccrine duct. It is not certain whether the cone cells form part of the epidermis or, as suggested by Lobitz *et al.* (1954), part of the "sweat duct unit". The rate of lumenal cell production within the intradermal duct is so much greater than the rate of prickle cell production within the cone region that the lumenal duct is forced to coil as it leaves the dermis and enters the cone.

The Control of Eccrine Duct Mitosis

Since epidermal mitotic activity is controlled by a chalone-dominated feedback system, it was first necessary to discover whether the epidermal chalone has any influence over the mitotic activity of the eccrine gland duct. In the first experiments, each mouse was given a single injection of a partially purified extract of either pig skin or pig palate. The doses given were known to be sufficient to induce an epidermal mitotic depression. The pig skin also contained sebaceous glands, and the skin extract therefore contained the sebaceous gland chalone; the palate extract did not contain any sebaceous gland chalone. Neither the skin nor the palate contained any eccrine sweat glands. The reactions of the epidermis, the sebaceous glands, and the eccrine ducts to these 2 extracts are shown in Table I.

TABLE I.—*The Effects of a Single Injection of Extracts of Skin and of Palate on the Mitotic Activity of Mouse Eccrine Gland Ducts, Epidermis, and Sebaceous Glands*

	Eccrine ducts			Epidermis			Sebaceous glands		
Injection of	$M \pm$ S.E.	N	Change	$M \pm$ S.E.	N	Change	$M \pm$ S.E.	N	Change
Control	$6 \cdot 1 \pm 0 \cdot 41$	16	—	$8 \cdot 6 \pm 0 \cdot 21$	15	—	$16 \cdot 7 \pm 0 \cdot 59$	15	—
0·4 mg pig skin extract*	$6 \cdot 0 \pm 0 \cdot 23$	20	−2%	$4 \cdot 7 \pm 0 \cdot 20$	15	−45%	$7 \cdot 3 \pm 0 \cdot 50$	26	−56%
Control	$5 \cdot 5 \pm 0 \cdot 42$	10	—	$10 \cdot 2 \pm 0 \cdot 23$	60	—	$14 \cdot 6 \pm 0 \cdot 57$	14	—
2 mg pig palate extract*	$5 \cdot 9 \pm 0 \cdot 27$	10	+7%	$8 \cdot 2 \pm 0 \cdot 30$	15	−20%	$14 \cdot 6 \pm 0 \cdot 51$	12	0

Average numbers of mitoses arrested by colcemid in 4 hours in sections cut 7 μm thick: eccrine ducts per 25 sections; epidermis per cm section length; sebaceous glands per 100 sections.

* Weight of a 71–80% ethanol fraction of an aqueous extract of pig skin or of pig palate.

These results show, first, that the epidermal chalone depressed the epidermal mitotic rate and that the sebaceous gland chalone depressed the sebaceous gland mitotic rate; and second, that neither extract, in the doses used, had any effect on the eccrine duct mitotic rate. It thus appears improbable that these chalones play any role in the control of eccrine duct mitotic activity.

The next question was whether the eccrine duct cells (and perhaps also the eccrine gland cells) synthesize their own specific chalone. However, no suitable skin samples containing eccrine glands were available in adequate quantity for extraction. The obvious choice would be human skin (which would also contain the epidermal and sebaceous gland chalones), but all fresh material available from operations is contaminated by antiseptics, which act as general mitotic inhibitors. An experiment was therefore carried out using injections of fresh human sweat. It was already known that active chalones are present in urine (Bullough and Laurence, 1971) and that when living granulocytes are washed in saline they readily release their granulocytic chalone (Rytömaa and Kiviniemi, 1968a, b). It was therefore considered possible that sweat might contain an eccrine gland chalone if such a substance exists. The results are in Table II.

TABLE II.—*Effect of a Single Injection of Human Sweat on the Mitotic Activity of Mouse Eccrine Gland Ducts, Epidermis, and Sebaceous Glands*

	Eccrine ducts			Epidermis			Sebaceous glands		
Injection of	M ± S.E.	N	Change	M ± S.E.	N	Change	M ± S.E.	N	Change
Control	7·6 ± 0·52	7	—	11·3 ± 1·15	7	—	13·9 ± 0·80	7	—
0·1 ml sweat	7·0 ± 0·84	7	−8%	11·6 ± 0·92	7	+3%	13·7 ± 1·16	7	−1%
0·5 ml sweat	5·7 ± 0·52	8	−25%	11·2 ± 0·82	8	−1%	13·1 ± 0·89	8	−6%
2·0 ml sweat	4·4 ± 0·34	8	−42%	11·1 ± 1·03	8	−2%	14·5 ± 1·16	8	+4%

Average numbers of mitoses arrested by colcemid in 4 hours in sections cut 7 μm thick; eccrine ducts per 25 sections; epidermis per cm section length; sebaceous glands per 100 sections.

These results show that an injection of human sweat causes a mitotic depression in the eccrine duct cells, which is proportional to the dose given, and that, even at high doses, such an injection has no significant effect on the mitotic activity of either the epidermis or the sebaceous glands. The sweat mitotic inhibitor is evidently specific in both its origin and its action, which is the first characteristic of a chalone.

The Stress Hormones and Eccrine Duct Mitosis

The second characteristic of the known chalones of the skin is that their antimitotic action is greatly strengthened in the presence of the stress hormones and especially of adrenalin. These hormones are not themselves antimitotic agents; they can inhibit mitosis only if enough endogenous chalone exists with which they can co-operate (Bullough and Laurence, 1964b).

Experiments were therefore performed to determine the effects on eccrine duct mitotic activity of single injections of adrenalin and of hydrocortisone. The results are in Table III.

TABLE III.—*Effect of a Single Injection of Adrenalin and of Hydrocortisone on the Mitotic Activity of Mouse Eccrine Gland Ducts, Epidermis, and Sebaceous Glands*

	Eccrine ducts			Epidermis			Sebaceous glands		
Injection of	M ± S.E.	N	Change	M ± S.E.	N	Change	M ± S.E.	N	Change
Control	7·7 ± 0·27	15	—	10·2 ± 0·23	60	—	16·4 ± 0·52	10	—
10 μg adrenalin	2·1 ± 0·19	15	−73%	3·0 ± 0·36	35	−71%	3·8 ± 0·40	10	−77%
Control	6·5 ± 0·53	10	—	10·2 ± 0·23	60	—	11·3 ± 0·89	15	—
10 μg hydrocortisone	6·7 ± 0·18	15	+3%	10·0 ± 0·58	15	−2%	10·0 ± 0·75	15	−12%
Control	8·2 ± 0·60	5	—	10·2 ± 0·23	60	—	11·3 ± 0·89	15	—
100 μg hydrocortisone	8·5 ± 0·73	5	+4%	9·8 ± 0·38	30	−26%	10·1 ± 0·59	15	−11%

Average numbers of mitoses arrested by colcemid in 4 hours in sections cut 7 μm thick: eccrine ducts per 25 sections; epidermis per cm section length; sebaceous glands per 100 sections.

These results confirm that an excess of adrenalin acts to inhibit mitotic activity in epidermis and sebaceous glands and show that its action on eccrine ducts is similar. In contrast, an excess of hydrocortisone produced either inconclusive or negative results; the analysis of hydrocortisone action in support of a chalone mechanism has so far been possible only *in vitro* (Bullough and Laurence, 1968b).

As a further check on the action of the stress hormones the effects of adrenalectomy were then tested. It was already known that the abnormal reduction in the stress hormone concentration following the loss of the adrenals greatly stimulates the mitotic rate of both the epidermis and the sebaceous glands (Bullough and Laurence, 1964b, 1970). The results obtained are in Table IV.

TABLE IV.—*Effect of Adrenalectomy on the Mitotic Activity of Mouse Eccrine Gland Ducts, Epidermis, and Sebaceous Glands*

	Eccrine ducts			Epidermis			Sebaceous glands		
After	$M \pm$ S.E.	N	Change	$M \pm$ S.E.	N	Change	$M \pm$ S.E.	N	Change
Control	$6 \cdot 5 \pm 0 \cdot 68$	5	—	$9 \cdot 5 \pm 0 \cdot 42$	35	—	$18 \cdot 4 \pm 1 \cdot 53$	9	—
1 day sham adrenalectomy	$4 \cdot 0 \pm 0 \cdot 31$	13	—	$8 \cdot 8 \pm 0 \cdot 74$	13	—	$13 \cdot 6 \pm 0 \cdot 95$	14	—
1 day adrenalectomy	$5 \cdot 1 \pm 0 \cdot 30$	12	+26%	$9 \cdot 9 \pm 0 \cdot 49$	15	+13%	$14 \cdot 1 \pm 0 \cdot 82$	15	+4%
2 day sham adrenalectomy	$5 \cdot 3 \pm 0 \cdot 22$	15	—	$7 \cdot 4 \pm 0 \cdot 77$	30	—	$17 \cdot 7 \pm 0 \cdot 83$	15	—
2 day adrenalectomy	$8 \cdot 5 \pm 0 \cdot 27$	14	+38%	$15 \cdot 8 \pm 0 \cdot 67$	30	+114%	$34 \cdot 5 \pm 1 \cdot 95$	14	+95%

Average numbers of mitoses arrested by colcemid in 4 hours in sections cut 7 μm thick: eccrine ducts per 25 sections; epidermis per cm section length; sebaceous glands per 100 sections.

The increase in the mitotic rates of all 3 test tissues is obvious, although within the period of the experiment the response of the eccrine ducts was less than that of the epidermis and of the sebaceous glands.

General Conclusions

These results indicate that the mitotic control mechanism in the eccrine ducts is probably tissue specific and that it is certainly distinct from those of the epidermis and the sebaceous glands. The eccrine gland mitotic control mechanism has similar characteristics to the other skin chalone systems: each skin chalone is tissue specific, it is produced within the same tissue on which it acts to inhibit mitosis, and its antimitotic action is greatly strengthened in the presence of adrenalin (which by itself is not antimitotic).

DISCUSSION

It has long been known that mitotic activity is rare in the eccrine gland cells, but, curiously, it has also been thought that " the cells of the duct . . . are normally mitotically inert " (Montagna, 1962; but see Christophers and Braun-Falco, 1967). Regarding the duct cells, the probable explanation is that most skin samples came from men who were awake, when the mitotic rate is normally low due to an excess of adrenalin (Bullough and Laurence, 1961). In contrast, the mice studied here were asleep, when the mitotic rate is high; they were also treated with colcemid.

The results indicate the existence of an eccrine gland chalone, and this agrees with the available evidence that each epidermal derivative has its own specific chalone control system or systems (Bullough and Deol, 1971). Whether there is more than one chalone in the eccrine gland is not yet clear. However, the gland is an organ, not a tissue, and it is therefore possible that there could be separate control systems in the gland and in the duct.

Clearly the intradermal and intraepidermal ducts are both parts of a single tissue, the cells of which show a form of stratification. As is usual in the epidermis and in epidermal derivatives, the mitotic cells are in contact with the dermis; the post-mitotic cells lie above them to form the functional lining of the duct lumen. Post-mitotic cells are always dying cells with a particular life expectancy (Bullough, 1967). During their lifetime the post-mitotic lumenal cells slide outwards over the firmly fixed basal cells until, having passed through the epidermis, they come to the surface and die.

The intraepidermal duct is formed of lumenal cells alone and therefore its wall is only one cell thick. However, Lobitz *et al.* (1954) have suggested that this duct is part of a larger unit in which must also be included a " periductal sheath " formed of 2 or more layers of epidermal-type prickle cells. These prickle cells are " smaller and poorer in glycogen than the adjacent prickle cells ", and they derive from basal cells which lie in the epidermal cone (or acrosyringium) and which " are smaller than the adjacent basal cells of the epidermis ". Lobitz *et al.* (1954) regard all these smaller basal and prickle cells as part of an " epidermal sweat duct unit ", which is " a biologic as well as a morphologic entity thatbe haves differently from the epidermis ".

The existence of these smaller basal and prickle cells within the cone region in man has been confirmed, and similar cells also occur in baboon skin (Bullough and Deol, 1972). However, in mouse footpads the cone cells do not appear to differ from the adjacent epidermal cells, and there is no obvious reason to regard them as part of the eccrine duct system. Even in man the cone cells react differently from the duct cells. Thus, in psoriasis the increased rate of epidermal cell production is also seen in the cone cells, but it is not matched by any increased rate of duct cell production. The consequence is that the intraepidermal duct straightens; conversely, epidermal hypoplasia also involves the cone cells and the duct then becomes supercoiled (Lobitz *et al.*, 1954). Thus the mitotic control-mechanism of the epidermis, including the cone region, seems to be independent of that of the eccrine duct.

At the lower end of the duct the ampulla presents another problem. With a lining epithelium only one cell thick it is difficult to envisage the fate of the post-mitotic cells. Possibly these cells are simply retained in the basal layer until they die and are lost into the lumen; alternatively, they may move outwards into the intradermal duct or inwards to replace lost gland cells.

As for the gland cells themselves it is certain that most, if not all, are post-mitotic, and the rarity of mitosis suggests that they must have a long functional life. The few mitoses seen in human skin could have been either in the gland cells themselves (Christophers and Braun-Falco, 1967) or in obscure cells that are few in number and that have remained mitotic. If they do occur in the functional gland cells, these cells must be able to revert to mitosis in the same way as can, for instance, the post-mitotic stratum spinosum cells when they suffer a fall in their intracellular chalone concentration after skin damage. The present evidence on the chalone content of the sweat suggests that a fall in the intracellular chalone concentration of the eccrine gland cells might occur as a consequence of active and prolonged secretion.

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THE EFFECT OF OCCLUSIVE AND SEMIPERMEABLE DRESSINGS ON THE MITOTIC ACTIVITY OF NORMAL AND WOUNDED HUMAN EPIDERMIS

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Summary.—Occlusion has been shown to increase the rate of closure of superficial wounds. However, it has also been shown to inhibit cell division both of psoriatic lesions and stripped skin. This work was undertaken to investigate further this apparent anomaly. Four dressings—an occlusive, adhesive tape; the adhesive backing from this tape; a less occlusive adhesive tape; and an occlusive, non-adhesive film, were used to cover regions of normal and stripped skin for periods from 2 to 6 days. The results show a slight increase in mitotic rate and epidermal thickness of normal skin, but a decreased mitotic rate and increased thickness of stripped skin, under all dressings. These results are discussed and it is suggested that the less occlusive films might be of benefit in accelerating the healing of wounds without adding the risk of infection seen under complete occlusion.

It has been demonstrated on many occasions that the rate of closure of superficial skin wounds can be increased by occluding the injured site. This is now known to be due to an increased rate of cell migration across the wound surface (Winter, 1962; Winter, 1967; Hinman and Maibach, 1963). However, during recent years Fry *et al.* (1970), using plastic film, and Baxter and Stoughton (1970), using Blenderm ® tape, showed that occlusion of psoriatic lesions would significantly reduce the mitotic activity of this hyperplastic epidermis; while Petzoldt *et al.* (1970) showed that occlusion with plastic film would also reduce much of the increased enzymatic activity found in these lesions. These results may, of course, be due to a peculiarity of the psoriatic epidermis, but Williams and Hunter (1957) showed inhibition of mitotic activity in skin stripped with cellophane tape followed by occlusion with the same tape; and Born (1969) demonstrated inhibition of DNA synthesis in normal skin after occlusion with plastic film. In view of this apparent anomaly, the present investigation was undertaken to document further the action of occlusive dressings on cell division in the normal and wounded epidermis.

MATERIALS AND METHODS

The basic technique used was that previously described by Fisher and Maibach (1971). The subjects used were normal adult males. Since several biopsies were required from each individual, investigation was confined to the back. Four different dressings were used: an adhesive, occlusive tape (Blenderm ®); the adhesive alone from this tape,

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presumably non-occlusive*; an adhesive, less occlusive, porous tape (Micropore ®); and a non-adhesive, occlusive, plastic film (Saran ® wrap—a chlorinated hydrocarbon polymer). These dressings were applied for 2–6 days, being carefully removed and replaced with fresh dressing applied daily. On the morning of the last day the dressings were removed, 0·5% Colcemid cream (*n*-desacetyl methyl colchicine) applied under an occlusive patch test plaster, for 6 hours, and 3 mm punch biopsies taken. Colcemid was employed to arrest cells at metaphase so that the greater number of mitoses present would simplify the counting and decrease the number of biopsies required. This basic experimental design allowed the use of each subject as his own control, which made the use of small groups of volunteers practical. The specimens were fixed in Davidson's fixative, embedded, cut at 7 μm and stained with the Feulgen reaction for DNA. Counts of dividing cells were made from 12 separate sections for each specimen and the mean mitotic rate expressed as mitoses per 1000 viable epidermal cells. Statistical significance was determined by the F test for analysis of variance (Goldstein, 1964). This test is similar to the Student t test, but was chosen because it is more flexible in its use and is better suited for highly variable systems such as that investigated in this study. In particular it allows for error such as might be found due to variation between individuals or between different counts from the same individual.

Experiment I

The adhesive occlusive tape was applied to normal skin for 3, 4 or 6 days and to stripped skin for 2, 3, 4 or 6 days. In the latter experiment the skin was stripped to glistening with cellophane tape prior to application of the dressing. On the last day of each experiment Colcemid cream was applied to each site under an occlusive patch test plaster. To determine whether the Colcemid was influencing the results, the 4-day experiment on normal skin was repeated without the application of this drug. In all instances the sites were paired, i.e. covered and open, on each individual, and also duplicated on the 2 sides of the back. Thus each individual acted as his own control.

Experiment II

To determine whether occlusion or adhesion, or both, were required to produce the observed effects, the following experiments were performed. A non-adhesive, plastic film; a porous, adhesive tape; and the adhesive alone from the tape used in the earlier experiments, were applied and renewed daily for either 3 or 6 days to both normal and stripped skin. Again, in both normal and stripped skin, one site remained uncovered as the control, and all sites were duplicated on both sides of the back. Colcemid cream was applied, under occlusion, to all sites on the last day of each experiment.

Experiment III

The dressings described above (excluding the adhesive alone) were also applied and renewed daily to 1 mm punch biopsy wounds. The purpose of this was to determine whether any chemical inhibitor might be present in the dressings. In a punch biopsy wound the increased mitotic activity seems to be secondary to cell migration into the wound (Van Winkle, 1968) and not due to a disturbance of the epidermal surface. However, the rapidly dividing cells are still easily accessible to any substance diffusing in from the surface. Thus any chemical inhibitor would show the same effect on the punch biopsy as on the stripped wound. In these studies the sites were anaesthetized with 1% lidocaine solution (without epinephrine), and full thickness skin was removed with a 1 mm punch. Haemostasis was obtained with pressure. When the area was dry (usually 1 hour later), the dressings were applied. The dressings were renewed daily for 3 days. On the last day, Colcemid cream was applied and 4 mm punch biopsies

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* Transfer tape No. PSL 48722

TABLE I.—*The Effect of an Occlusive Adhesive Tape on the Mitotic Activity of Normal and Stripped Skin*

	Normal					Stripped				
	Treated		Control			Treated		Control		
Time (days)	*N*	Mitotic index ± S.E.	*N*	Mitotic index ± S.E.	*P*	*P*	Mitotic index ± S.E.	*N*	Mitotic index ± S.E.	*P*
2	—	—	—	—	—	43	3·36±0·21	48	18·65±2·05	<0·005
3	78	0·75±0·14	78	1·06±0·15	>0·05	48	3·91±0·58	48	19·60±0·90	<0·005
4	192	1·24±0·12	191	0·62±0·06	<0·005	48	9·62±1·20	48	17·30±0·94	<0·005
No colcemid	48	0·15±0·04	48	0·07±0·02	>0·05	—	—	—	—	—
6	78	0·65±0·08	78	0·65±0·07	>0·05	48	1·04±0·16	47	5·15±0·76	<0·005

N = total number of individual slides counted—usually 6 per biopsy.

TABLE Ia.—*The Effect of Occlusive Adhesive Tape on the Epidermal Depth* of Normal and Stripped Skin*

	Normal					Stripped				
	Treated		Control			Treated		Control		
Time (days)	*N*	Cells/unit Length ± S.E.	*N*	Cells/unit Length ± S.E.	*P*	*N*	Cells/unit Length ± S.E.	*N*	Cells/unit Length ± S.E.	*P*
2	—	—	—	—	—	86	89·0±2·59	96	87·1±2·01	<0·05
3	156	78·1±1·91	156	72·0±1·51	<0·025	96	102·6±2·80	96	79·9±1·75	<0·005
4	480	81·9±1·00	478	76·1±1·10	<0·005	96	127·1±5·06	96	91·5±2·30	<0·005
6	156	76·4±1·83	155	58·8±3·63	<0·005	96	89·0±2·80	94	83·1±2·83	<0·005

* The epidermal depth was measured in terms of viable cells per unit length of epidermis. The unit of length was arbitrarily taken as the width of an eyepiece grid. Cells were counted throughout the complete epidermal depth staying within these lateral borders.

N = total number of counts made—usually 12 per biopsy.

taken 6 hours later, so as to remove the earlier wound site *in toto*. Also in this experiment the wound size was determined by measuring the distance between the opposing epithelial edges. This was done with an eyepiece micrometer.

RESULTS

Occlusive Tape

The occlusive adhesive tape was arbitrarily chosen as the standard dressing, and its effect on the mitotic activity of both normal and stripped skin was investigated with respect to time.

As noted in Table I, occlusive tape applied to normal skin produced no significant change in mitotic activity at either 3 or 6 days. However, at 4 days, there was a significant doubling of the mitotic rate at the occluded site, compared to the normal skin. This was rechecked in additional experiments not employing Colcemid. An increase in mitotic count was again observed, although on this occasion it was not significant, possibly due to the smaller numbers involved, since fewer mitoses are seen without Colcemid.

Quite a different effect occurred in skin whose mitotic rate was greatly increased by removal of the stratum corneum with cellophane tape stripping (Table I). When examined at 2, 3 and 4 days after stripping, the stripped untreated sites had a 17-fold or greater mitotic rate than normal skin. Occlusive tape application markedly decreased the mitotic rate to much closer to normal levels. Six days after stripping, the mitotic rate of the untreated sites was still approximately 5 times higher than normal skin, but that of the occluded sites was now reduced to normal levels, In other words, the very high rate of cell division induced by stripping is, in the main, inhibited.

On examining these sections, we noted that the epidermal thickness seemed to be different under the occlusive tape. Consequently, epidermal thickness was measured, using an eyepiece micrometer. An arbitrary unit length of epidermis was taken as that length found between the borders of an eyepiece grid. All viable cells were then counted under oil immersion, throughout the depth of the epidermis, but within the lateral boundaries of the grid. This type of measurement is greatly dependent upon the ability to obtain perfectly transverse sections. Care was taken in this regard; also, since the material was prepared without knowledge of the identity of each specimen, bias was unlikely to enter into the procedure. Furthermore, since only a comparison between different treatments was required, and not quantitative measurements, use of a good statistical method (Goldstein, 1964) would identify any significant change in epidermal thickness. As can be seen from Table Ia, there is a minimal, but definite increase in the thickness of normal skin which has been occluded with tape. A greater increase in thickness occurred in stripped skin which had been so treated.

Other Dressings

These effects were then compared with similar applications of the other dressings at 3 and 6 days. The data are in Table II. At 3 days on normal skin the adhesive mass from the occlusive tape showed no appreciable effect. However, the less occlusive, " porous " tape and the occlusive, plastic film produced definite reductions in mitotic rate. Similar effects were found after 6 days. At both

TABLE II.—*The Effect of Various Dressings on the Mitotic Activity of Normal and Stripped Skin*

		Normal			Stripped		
Time (days)	Dressing	*N*	Mitotic index ± S.E.	*P*	*N*	Mitotic index ± S.E.	*P*
3	Occlusive adhesive tape	78	0·75±0·14	>0·05	48	3·91±0·58	<0·005
	Plastic film (no adhesive)	71	0·65±0·08	<0·025	48	4·93±0·62	<0·005
	" Porous " tape	78	0·22±0·03	<0·005	44	0·91±0·17	<0·005
	Adhesive mass (without occlusion)	78	0·92±0·18	>0·05	48	2·11±0·47	<0·005
	Control (untreated skin)	78	1·06±0·15	—	48	19·60±0·90	—
6	Occlusive adhesive tape	78	0·65±0·08	>0·05	48	1·04±0·16	<0·005
	Plastic film (no adhesive)	78	0·44±0·05	<0·025	47	1·87±0·15	<0·005
	" Porous " tape	72	0·29±0·04	<0·005	46	10·56±1·51	<0·005
	Adhesive mass (without occlusion)	74	0·67±0·09	>0·05	46	2·51±0·63	<0·01
	Control (untreated skin)	78	0·65±0·07	—	47	5·15±0·76	

3 and 6 days the greatest reduction in mitotic rate occurred with the less occlusive, " porous " tape.

On stripped skin all materials (including the adhesive mass) produced a significant reduction in mitotic rate at 3 days. Generally, a greater inhibition was observed under the less occlusive dressings (adhesive mass and " porous " tape) than under the occlusive dressings (adhesive, occlusive tape and occlusive film), although the " porous " tape consistently showed the greatest effect. However, at 6 days, when the mitotic rate of the control sites was returning towards normal, those sites covered with the " porous " tape once more showed high counts. The other treated sites still remained lower than the controls.

TABLE IIa.—*The Effect of Various Dressings on the Epidermal Depth* of Normal and Stripped Skin*

		Normal			Stripped		
Time (days)	Dressing	*N*	Cells/unit Length ± S.E.	*P*	*N*	Cells/unit Length ± S.E.	*P*
3	Occlusive adhesive tape	156	78·1±1·91	<0·025	96	102·6±2·80	<0·005
	Plastic film (no adhesive)	142	87·4±3·27	<0·005	96	105·8±2·64	<0·005
	" Porous " tape	156	76·1±1·88	>0·05	88	99·4±3·27	<0·005
	Adhesive mass (without occlusion)	156	84·9±2·35	<0·005	96	98·6±2·60	<0·005
	Control (untreated skin)	156	72·0±1·51	—	96	79·9±1·75	—
6	Occlusive adhesive tape	156	76·4±1·83	=0·05	96	89·0±2·80	<0·005
	Plastic film (no adhesive)	156	81·2±1·91	<0·005	94	100·4±2·92	<0·005
	" Porous " tape	144	76·3±1·70	<0·05	94	119·0±3·83	<0·005
	Adhesive mass (without occlusion)	148	85·5±2·38	<0·005	94	105·5±3·25	<0·005
	Control (untreated skin)	155	71·7±1·50	—	94	83·1±2·83	—

* The epidermal depth was measured in terms of viable cells per unit length of epidermis.

As can be seen from Table IIa, there was minimal, but significant increase in thickness of normal skin from these dressings and a much bigger increase after application to stripped skin.

Punch Biopsy Wounds

The effect of covering a punch biopsy wound for 3 days is seen in Table III. A minimal increase in mitotic activity was found with the occlusive tape (compare the effect on normal skin), but otherwise there was no effect.

In the same experiment the rate of epithelialization was noted by measuring the distance between the 2 opposing epithelial edges. As can be seen from Table III, the rate of closure under each of the 3 dressings was approximately double that of the control. However, the occlusive tape also showed a significantly smaller wound size than either the plastic film or the " porous " tape.

Table III.—*The Effect of Various Dressings on* 1 *mm Punch Biopsy Wounds*

Time (days)	Dressing	N	Mitotic index ± S.E.	P	N	Wound size* ± S.E.	P
3	Occlusive adhesive tape	45	$9\cdot04 \pm 0\cdot93$	$>0\cdot05$	84	$1\cdot06 \pm 0\cdot05$	$<0\cdot005$
	Plastic film (no adhesive)	43	$7\cdot34 \pm 0\cdot63$	$>0\cdot05$	73	$1\cdot21 \pm 0\cdot05$	$<0\cdot005$
	" Porous " tape	35	$7\cdot65 \pm 1\cdot49$	$>0\cdot05$	82	$1\cdot23 \pm 0\cdot05$	$<0\cdot005$
	Control (untreated skin)	43	$7\cdot18 \pm 0\cdot65$	—	81	$1\cdot85 \pm 0\cdot07$	—

* This was measured in terms of micrometer eyepiece units as the distance between the opposing epithelial edges.

The occlusive tape shows a significantly smaller wound size then either the plastic film or " porous " tape. $P < 0\cdot005$.

DISCUSSION

Since basically no effect of these dressings was found on the mitotic activity at the edge of a circular punch biopsy wound after 3 days, it is not likely that there is any chemical inhibitor present in these tapes. It is interesting to note that occlusion does indeed decrease the time required for wound closure, as already shown by Winter (1967) in the pig and Hinman and Maibach (1963) in man. However, we have shown here that the same effect can be observed under a relatively non-occlusive tape (Micropore®). The pores in this tape are those found only within the weave of the material. Since these are particularly small, one might suggest a " partial occlusion "—something between a polyethylene film and the perforated plastic dressing (Telfa) investigated by Winter (1965). However, when a dressing of this type is covering wounded skin, absorption of the exudate would probably make it more occlusive.

Since we have thus confirmed the increased healing rate seen under occlusion, how does one explain the effects seen on cell division? On investigating the effects of essential fatty acid (EFA) deficiency, Menton (1968) suggested that the resultant epidermal hyperplasia may have been due to an abnormal stratum corneum and hence an abnormal water barrier, with increased epidermal permeability. On putting EFA-deficient mice, with their abnormal stratum corneum, into a humid environment, he did indeed show that the degree of hyperplasia was reduced. Since, in stripped skin, water loss is excessive, any type of covering will reduce this. Consequently, if there is a connection between the integrity of the epidermal water barrier and epidermal proliferation, one might expect the results demonstrated in this presentation. Thus, in excised wounds, one would expect increased epithelialization under occlusion; but, since the water loss across the new epithelium is reduced, the subsequent mitotic activity and the resultant epidermal thickening would also be reduced. This is in accord with the results shown by Gillman (1958). A second possibility is that there is a negative feedback existing between the keratinized layers and the basal layer which maintains a steady state in epidermal depth. This has been suggested many times in theory, but the only evidence for such a concept is that of Born and Bickhardt (1968), who suggested that extracts of the stratum corneum would inhibit DNA synthesis in the epidermis.

The return of stripped skin to high levels of mitotic activity after 6 days covering only with the " porous " tape is difficult to explain. However, since the dressings were changed daily, it is possible that there was a renewed stripping effect if insufficient care was taken, and absorption of exudate by the " porous " tape might result in greater adherence to the wound surface. Pinkus (1952) showed that very little stripping is needed to increase mitotic activity. In fact, the extraordinarily wide range of results in this particular instance (0·23–24·21) might support such an explanation.

Since the reduction in mitotic activity is particularly marked in stripped skin which has been covered, how does one explain the equally marked increase in epidermal thickness under identical circumstances? A possible explanation is that, since keratinization requires exposure to the ambient environment, and as this is excluded by the dressing, the increased thickness is simply representative of viable cells which, under other conditions, would have formed part of the stratum corneum. On this basis one might expect increased thickness in the stripped covered skin, since even the reduced mitotic rate is still above normal.

In conclusion, one can see that all the dressings investigated in this report produce a decrease in the epidermal mitotic response after stripping, and increased cell migration across an excised wound. It is emphasized that these effects do not seem to be dependent upon complete occlusion; consequently the less occlusive dressings may have some advantage, since the risk of infection would be reduced.

These results suggest that all the types of dressing used in this study produce a change in the local environment such as to decrease the trans-epidermal water loss, increase cellular migration and possibly decrease keratinization. These factors in turn are probably affecting cell division by some indirect mechanism as yet unknown.

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NUCLEAR AND NUCLEOLAR ULTRASTRUCTURE DURING THE LATE STAGES OF NORMAL HUMAN KERATINOCYTE MATURATION

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AND W. OHELERT

Summary.—Ultrastructural changes of nuclei and nucleoli were studied during the physiological maturation of human keratinocytes. Most nuclear and nucleolar changes were observed in keratinocytes of the upper cell layer of the stratum granulosum, in which these cells are transformed to horny cells. Nucleolar changes were characterized by the segregation of nucleolar ribonucleoprotein components, the formation of ring shaped nucleoli and by a reduced quantity of granular components. In addition to the known nuclear ultrastructural changes in keratinocytes of the upper cell layer of the stratum granulosum, an increased number of perichromatin granules was noted in the nuclei of these cells. The number of interchromatin granules was variable.

The nucleolar segregation, formation of ring-shaped nucleoli and the decrease of nucleolar granular components in keratinocytes of the upper cell layer of the stratum granulosum, together with the increase in the number of perichromatin granules, might suggest that the RNA synthesis in these cells is inhibited first in the nucleoli before the cessation of extranucleolar RNA synthesis and nuclear disintegration.

NUCLEAR and nucleolar changes during cell maturation and differentiation have been the subject of numerous reports in the literature (Busch and Smetana, 1970; Smetana and Potměšil, 1968; Smetana *et al.*, 1970a). The ultrastructural changes in the nucleus and nucleolus during the physiological differentiation and maturation of keratinocytes of the human epidermis have received little attention, although these cells represent a convenient model for such studies. Keratinocytes proliferate in the basal and suprabasal layers of the stratum germinativum. In more superficial layers the keratinocytes mature and finally transform into cornified cells. Changes in the ultrastructure of nuclei and nucleoli in these last stages of the maturation of keratinocytes are as yet not well known (Odland and Reed, 1967).

To provide more information on nuclear and nucleolar ultrastructure in normal human keratinocytes during the final stages of physiological maturation, these cells have been studied by conventional procedures.

MATERIAL AND METHODS

The normal human skin used in this study was removed under local procaine anaesthesia from the forearm and the back of 11 normal individuals. The tissues were cut into small

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blocks, fixed for 4 hours in a 2·5% glutaraldehyde solution, buffered at pH 7·2 with phosphate buffer, and left overnight in the same buffer. Some blocks from each sample were postfixed in 1% osmium tetroxide in 0·1 mol/l phosphate buffer for 2 hours. All of these procedures were performed at 4°C. Following the dehydration in graded series of ethanol, the blocks were embedded in either Epon or Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with Siemens Elmiskop IA and Tesla 613 electron microscopes.

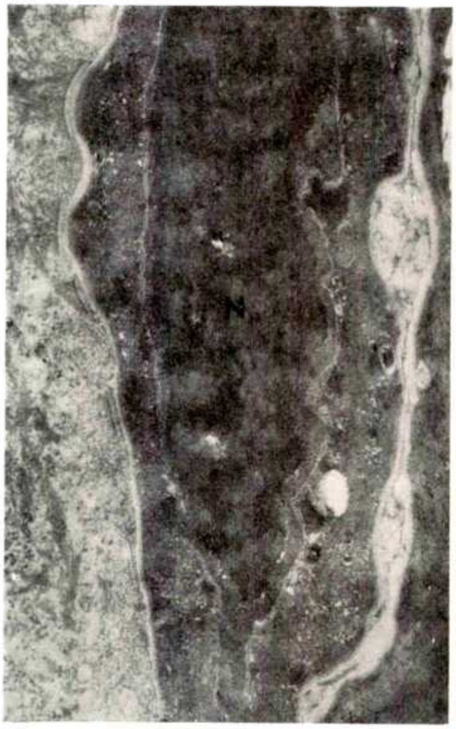

FIG. 1.—Persistent nuclear remnants (N) in the lowermost layer of the stratum corneum. × 26,000.

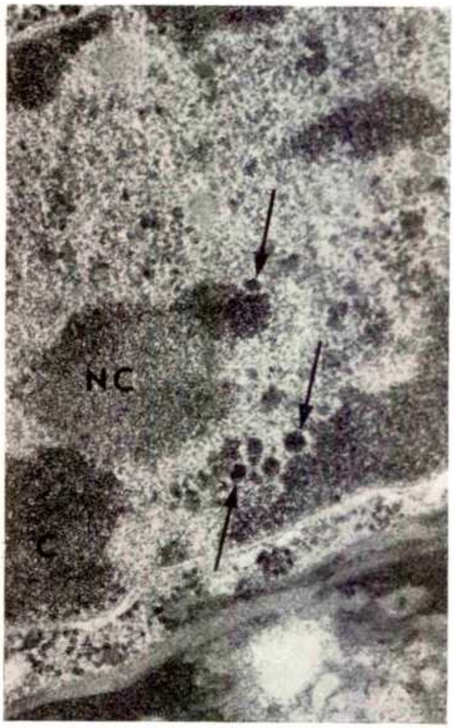

FIG. 2.—Part of a flattened nucleus with a moderately undulating surface. Condensed chromatin (C), nucleolus (NC), perichromatin granules (arrows). × 50,000.

RESULTS

The ultrastructural morphology of the nuclei and nucleoli of the keratinocytes of the lower cell layers of the stratum granulosum was found not to differ substantially from that of the keratinocytes of the stratum spinosum. Distinct changes in the nuclei and nucleoli have been observed in keratinocytes of the upper cell layer of the stratum granulosum in which the keratohyaline granules accumulate and most of the cytoplasmic organelles, as well as the nuclei, undergo physiological disintegration and presumably disappear (Odland and Reed, 1967 Wier *et al.*, 1971). However, very few nuclear remnants persist in the lower cell layer of the stratum corneum (Fig. 1).

The nuclear ultrastructure of keratinocytes in the upper cell layer of the stratum granulosum was found to be variable. Most nuclei are flattened; they have moderately undulating surfaces and usually one deep cytoplasmic invagination (Fig. 3). The less frequently observed rounded nuclei have an irregular outline.

The chromatin in most nuclei of the upper cell layer of the stratum granulosum is characterized by condensations at the periphery of the nucleus, as well as throughout the nucleus (Fig. 2, 4, 5). A striking increase of the quantity of perichromatin granules was observed in flattened as well as in rounded irregular nuclei. The size of these granules was found to be variable, ranging from 15 to 50 nm (Fig. 2). In some nuclei numerous interchromatin granules were also observed (Fig. 3). Furthermore, flattened nuclei occasionally were found to contain conglomerations of granules that were similar in size and shape to small perichromatin granules (Fig. 4). We found that the ultrastructure of both the perichromatin and the interchromatin granules did not differ from the earlier descriptions of Bernhard and Granboulan (1968) and Monneron *et al.* (1968). It was observed that flattened nuclei also frequently contain spherical granules and bodies of various density and size (Fig. 4, 5), some being similar to the kerato-

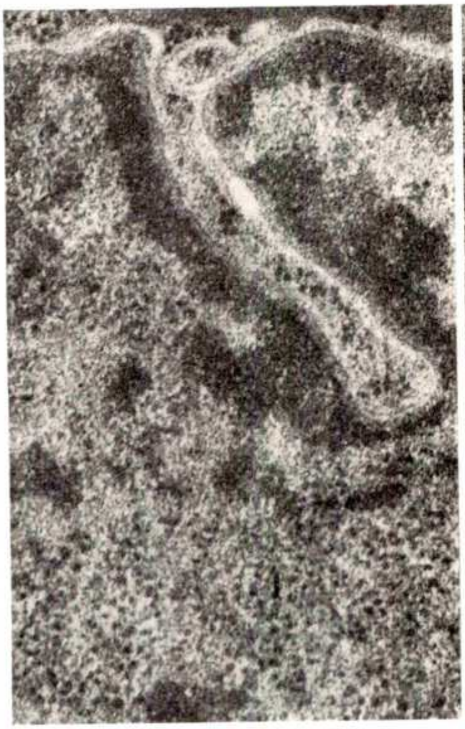

FIG. 3.—Higher magnification of part of Fig. 2. Note the large number of interchromatin granules (I). × 55,000.

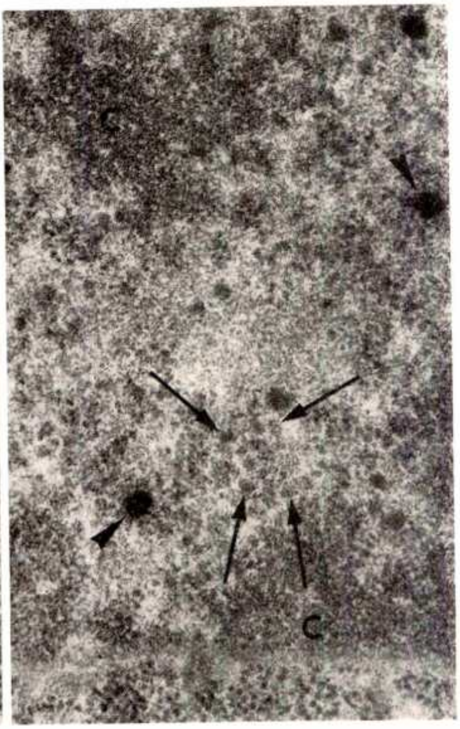

FIG. 4.—A nuclear portion of a keratinocyte containing large dense granules (point) and clusters of granules, presumably representing perichromatin granules (arrows). Condensed chromatin (C). × 50,000.

hyaline-like inclusions previously described in these nuclei (Matoltsy and Parakkal, 1967; Wier *et al.*, 1971).

Most nucleoli in keratinocytes of the upper cell layer of stratum granulosum are compact, with no distinct nucleolonemata. Ring-shaped nucleoli and nucleoli with nucleolonemata were less frequent. Small compact nucleoli are usually characterized by the segregation of granular and fibrillar components (Fig. 6). In addition, a few such nucleoli contained " fibrillar centres " (Recher *et al.*, 1970), which were located at the nucleolar periphery (Fig. 7). Dense, unusual, large granules in nucleoli, with segregation of ribonucleoprotein components, were also noted (Fig. 8), although infrequently. Ring-shaped nucleoli are composed of a shell surrounding a light central core (Fig. 9). The distribution of nucleolar components in these nucleoli, as well as their ultrastructure, was similar to that described in other cell types (Smetana *et al.*, 1970a; Smetana *et al.*, 1970b).

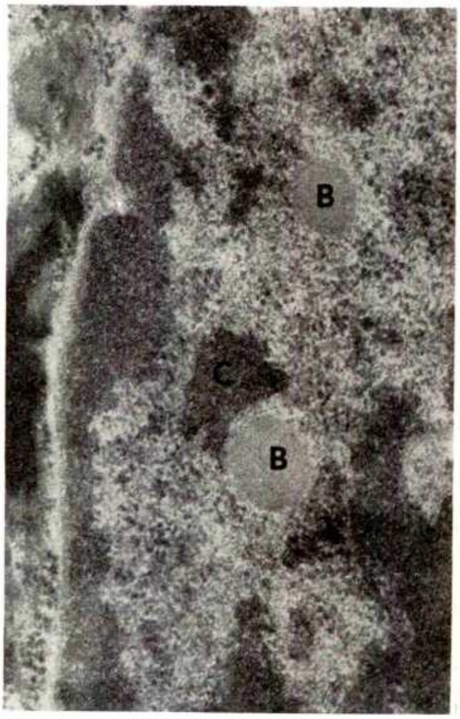

FIG. 5.—Large bodies (B) of moderate density in a nucleus of a keratinocyte with condensed chromatin (C). × 50,000.

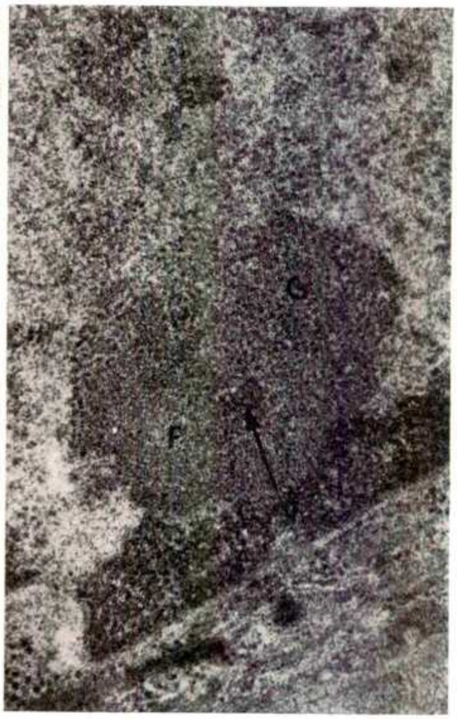

FIG. 6.—Distinct segregation of nucleolar components. Granular components (G), fibrillar components (F), intranucleolar chromatin (arrow). × 50,000.

The ultrastructure of nucleoli with nucleolonemata did not appear to differ from that of other cell types (Bernhard and Granboulan, 1968; Busch and Smetana, 1970). However, some of these nucleoli consist mainly of fibrillar components. Light areas in such nucleoli are usually small, giving these nucleoli a relatively compact appearance (Fig. 10).

DISCUSSION

The present study provides new additional information on the nuclear and nucleolar morphology of human keratinocytes during the final stages of maturation. Most of the changes in nuclear and nucleolar maturation were noted in keratinocytes of the upper cell layer of the stratum granulosum, in which these structures undergo physiological disintegration (Odland and Reed, 1967).

The nucleolar changes were characterized by the segregation of nucleolar components, ring-shaped appearance and decrease in the amount of granular components. The segregation of nucleolar components was similar to that produced under experimental conditions by some inhibitors of RNA synthesis (Bernhard and Granboulan, 1968; Busch and Smetana, 1970; Simard and Bernhard, 1966). However, the nucleolar segregation observed in the present study was induced by the physiological cell maturation and differentiation, since the subjects studied had not been treated in any way. Ring-shaped nucleoli in keratinocytes were also similar to those induced by actinomycin D (Panteleakis and Mateyko, 1966; Smetana and Potměšil, 1968), as well as by cell maturation during which the RNA synthesis was apparently reduced (Busch and Smetana, 1970; Smetana *et al.*, 1970a; Smetana *et al.*, 1970b). Thus, both the segregation of nucleolar components

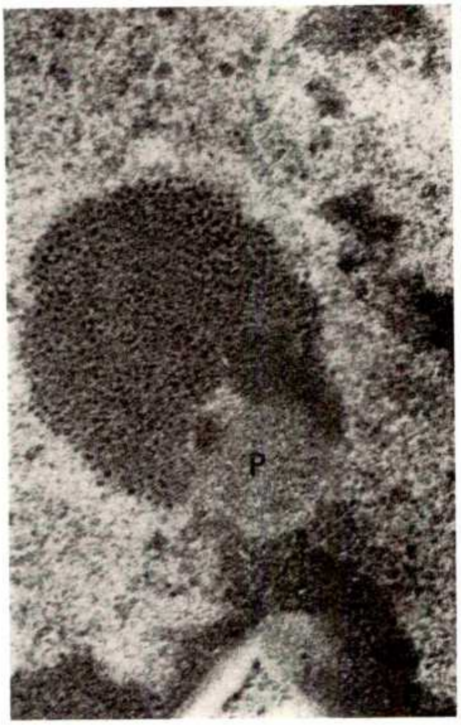

FIG. 7.—Small nucleolus with segregation of nucleolar components and prominent fibrillar centre (P). × 48,000.

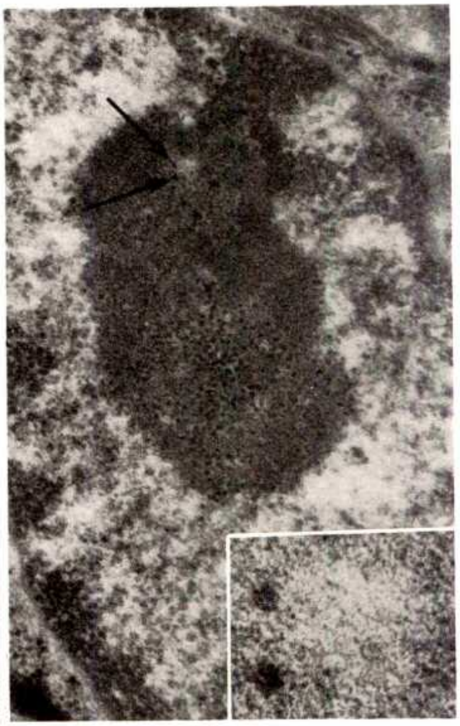

FIG. 8.—A nucleolus with segregation of nucleolar components and atypical dense granules (arrows and the inset). × 32,400. Inset × 222,000.

and the formation of ring-shaped nucleoli in keratinocytes of the upper cell layer of the stratum granulosum indicate a decrease of nucleolar functional activities in the production of new RNA-containing structure in these cells as the result of their physiological maturation.

The present study also demonstrated the presence of large dense granules in the segregated nucleoli of keratinocytes in the most superficial layer of the stratum granulosum. These granules are similar to those found in various cells after fixation with osmium tetroxide or glutaraldehyde with collidin buffer (Sankaranarayanan and Busch, 1965; Shinozuka, 1970; Verbin *et al.*, 1969). In the present study, however, the specimens were fixed in glutaraldehyde and post-fixed in osmium tetroxide buffered with phosphate. Since the presence of large dense granules in segregated nucleoli of keratinocytes was rare, no conclusions can be drawn concerning their morphology or their role and composition in these cells.

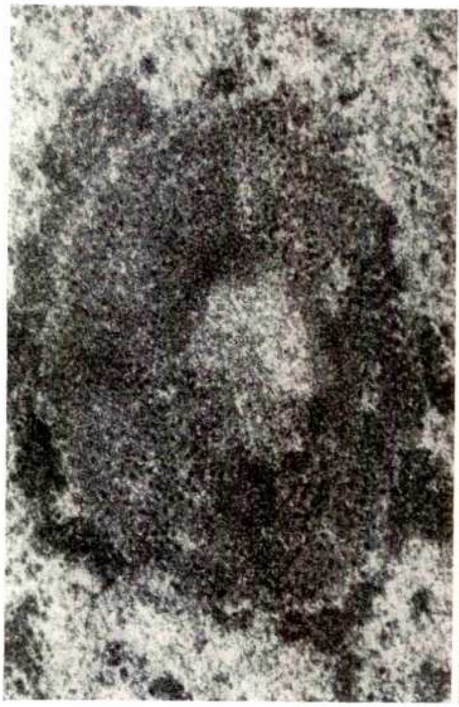

FIG. 9.—A ring-shaped nucleolus. × 50,000.

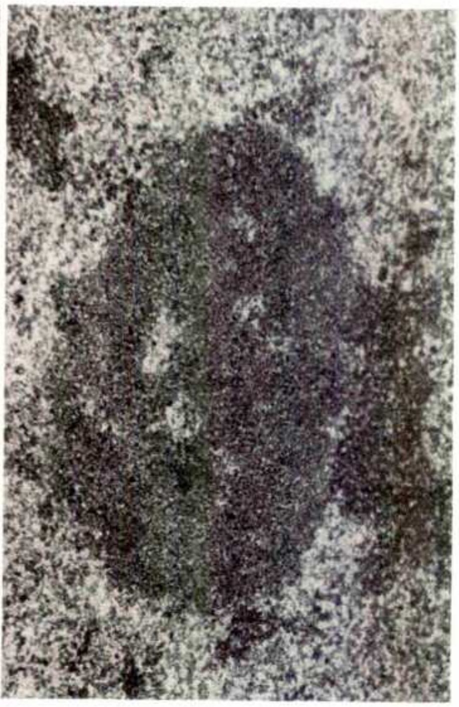

FIG. 10.—A nucleolus with nucleolonemata × 34,000.

The principal changes in nuclear ultrastructure observed in the keratinocytes of the upper cell layer of the stratum granulosum were characterized by chromatin condensation and an increase in the quantity of perichromatin granules. The condensation of the chromatin, together with deep cytoplasmic invagination into nuclei and the undulation of their surface seen in the electron micrographs of maturing keratinocytes, correspond to the pyknotic appearance of the nuclei in these cells, which is known from light microscopy. Such changes seem to indicate gradual regressive changes in nuclei which, in the upper cell layer of the stratum granulosum, disintegrate and finally disappear (Odland and Reed, 1967; Wier *et al.*, 1971). The chromatin condensation might be related to its inactivation produced by the cell maturation as described in other cell types (Ringertz, 1969; Ringertz *et al.*, 1970).

The increase in the number of perichromatin granules or the presence of conglomerations of such granules are difficult to explain. However, increase in the numbers of similar granules has been observed after the experimental inhibition of the RNA synthesis by actinomycin D, aflatoxin or lasiocarpine (Monneron and Kerros, 1970; Monneron *et al.*, 1968; Smetana and Potměšil, 1968). In such experiments the synthesis of nucleolar RNA was inhibited, in contrast to the production of the extranucleolar RNA, which seemed to continue (Monneron and Kerros, 1970). Thus, the increased number of perichromatin granules, together with nucleolar changes discussed above, might suggest that the synthesis of RNA during the maturation of the human keratinocyte is repressed in the nucleoli before it ceases in the extranucleolar regions of the nucleus.

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FLUORIMETRIC ASSAY OF CARBOXYLIC ESTER HYDROLASES IN GUINEA-PIG SKIN AND THE EFFECTS OF PROPRANOLOL

D. W. K. COTTON AND JOSÉ J. M. A. VAN DEN HURK

Summary.—The application of a continuous fluorimetric assay technique to carboxylic ester hydrolases (including the cholinesterases) in skin is described.

The kinetic properties of guinea-pig skin preparations from different anatomical sites are reported.

The *in vitro* and *in vivo* effects of propranolol and some other pharmacological compounds on carboxylic ester hydrolases from guinea-pig skin are described.

The results are discussed in relation to atopy.

It has been observed that intradermal injections of acetylcholine in atopic subjects produce a greater total sweat response than in normal subjects (Warndorff, 1970) and that simultaneous administration of propranolol in normal subjects shifts this response in the direction of the atopic (Hemels, 1970). This latter finding has been criticized by Foster *et al.* (1971). However, Hemels's results were calculated as a function of total sweat volume and Foster's results were calculated in terms of the maximum sweat rate (the peak height of the response). Both sets of workers assume that the effects of propranolol are mediated by a blockade of the β adrenergic receptor; but, as we have pointed out previously, some other mechanisms may be involved (Cotton and Sutorius, 1971).

The interest in the effects of propranolol on sweating skin stems from the work of Szentivanyi (1968) who suggested an endogenous β blockade in the atopic state. We have previously criticized this suggestion on a number of grounds and have pointed out that a large variety of abnormalities in the control mechanisms of cell metabolism could produce effects similar to those that Szentivanyi describes as a consequence of β blockade (Cotton, 1971; Mier, 1971).

The most obvious route by which propranolol could affect the sweating process, other than β blockade, is by influencing the enzymes responsible for the metabolism of the physiological mediators of sweating. The best known of these mediators is acetylcholine, and we have therefore studied the carboxylic ester hydrolase (3.1.1) activity in guinea-pig skin. To do this we have adapted a cholinesterase assay (Guilbault and Kramer, 1965) for use in skin. In this paper we describe the assay procedure, the enzyme activity in guinea-pig skin and the effects of propranolol.

MATERIALS AND METHODS

Fluorimeter.—The instrument used was the Baird Atomic " Fluorispec " S.F.-100 connected with a Servogor flat-bed recorder RE 511. The " Fluorispec " was fitted with a constant temperature cuvette holder, and the assays were carried out at 37°C. The settings of the Fluorispec were: slit 1 minimum, slit 4 maximum; slits 2 and 3 are

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not used with the constant temperature cuvette holder. The coarse gain of the Fluorispec was used together with the sensitivity control of the recorder to give initial velocity recordings of approximately 45° on the chart paper. The results were then recalculated to the settings used for the calibration graphs.

Guinea-pigs.—Female, adult, albino animals (Cp6-TNO) were used. They were killed by a blow to the neck. Skin was dissected off, scraped free of underlying connective tissue and muscle, minced with scissors and homogenized in buffer with a Townson and Mercer micro-wet grinder fitted with an ice jacket.

Chemicals.—These were Merck analytical grade, and the bovine erythrocyte acetylcholinesterase and β-naphthyl acetate were purchased from Sigma.

The various medicaments tested as inhibitors were obtained from the hospital pharmacy in their usual form and were used without any prior purification, except for the aspirin, which was pure acetylsalicylic acid. The propranolol used was Inderal I.C.I.

Assay method.—The procedure was essentially that of Guilbault and Kramer (1965) using β-naphthyl acetate as substrate. This is split by carboxylic ester hydrolases, such as acetylcholinesterase (3.1.1.7) and cholinesterase (3.1.1.8), to acetate and β-naphthol, and the reaction can be followed by the increasing fluorescence due to β-naphthol. The reaction was carried out in tris buffer at pH 7·5 with excitation 315 nm and fluorescence 420 nm. The incubation mixtures consisted of the substrate and enzyme, or skin homogenate, in buffer brought to a final volume of 0·5 ml with buffer.

EXPERIMENTAL

Standard curve for β-naphthol fluorescence.—β-Naphthol was dissolved in 0·05 mol/l tris buffer pH 7·5 containing 5×10^{-4} mol/l EDTA and 3×10^{-2} mol/l $MgCl_2$ and a standard curve prepared in the range 0·01–1 μg/ml.

Linearity with enzyme concentration.—A solution of purified acetylcholinesterase was made up in buffer (1 mg/ml) and various aliquots assayed. A similar experiment was performed using an homogenate of guinea-pig skin.

pH optimum.—A skin homogenate prepared in water was assayed at various pH values between 4·5 and 9·0 at 3 different substrate concentrations: 269 μmol/l, 484 μmol/l and 806 μmol/l.

Kinetics.—The enzyme was assayed over a range of substrate concentrations (200–800 μmol/l) in skin from various regions of the guinea-pig: head, ear, back, upper and lower limbs, and foot pads.

Inhibitors in vitro.—The effects of prostigmine, aspirin, methotrexate, atabrine, propranolol, regitin and isoprenaline were studied on the purified bovine erythrocyte enzyme and on the skin preparations over a range of substrate concentrations (200–800 μmol/l). The pharmacological agents were used in a final concentration of 20 μg/ml. Propranolol, prostigmine and isoprenaline were studied over a range of final concentrations: 20–200 μg/ml.*

Propranolol in vivo.—Six guinea-pigs were shaved on each flank, and on the left flank were injected intradermally with 0·01 mg propranolol in 0·1 ml saline. On the right flank they were injected with 0·01 ml of saline as a control. The animals were killed at 0, 0·5, 1, 2, 6 and 24 hours after injection; the flank skin was removed for about 1 cm² around the injection site and the enzymatic activity was assayed over the range of substrate concentrations 200–800 μmol/l.

RESULTS

Standard curve for β-naphthol fluorescence.—The plot of fluorescence against quantity of β-naphthol was linear over the range 0·01–1 μg/ml and sensitive to below 0·01 μg/ml.

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* Prostigmine = Neostigmine, Atabrine = Mepacrine, Regitin = Phentolamine.

Linearity with enzyme concentration.—The initial velocity was linear u 0·3 mg/ml purified bovine erythrocyte enzyme final concentration and u 5 mg/ml skin homogenate final concentration.

pH optimum.—The pH optimum for the skin enzyme was between 7·5 and but additionally showed an increase with decreasing pH, presumably due to hydrolysis of the substrate. The apparent $V_{\max}$ increased with decreasing and the apparent Km decreased; thus these kinetic values are pH-dependent this assay method.

Kinetics.—The enzyme in skin from various regions gave a wide spread of and $V_{\max}$ values (Table I).

TABLE I.—*Kinetic Variability with Site*

Skin site	Km 10^{-6} mol/l	$V_{\max}$ μmol/min/g wet wt.
Flank	769	0·351
Flank	800	0·532
Flank	606	0·392
Flank	518	0·375
Flank	596	0·340
Flank	704	0·372
Scalp	319	0·013
Lower hind leg	157	0·050
Upper hind leg	313	0·020
Ear	2000	0·180
Upper fore leg	625	0·289
Lower fore leg	2000	0·122
Hind foot pad	4000	0·205

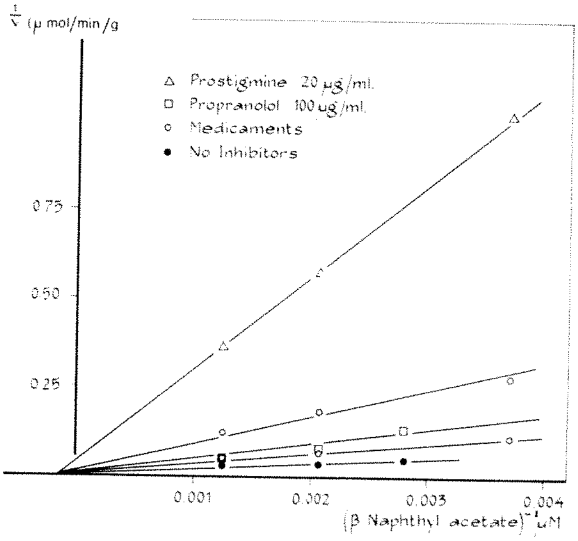

FIG. 1.—Lineweaver and Burke plots of purified bovine erythrocyte acetylcholinesterase with and without various medicaments and inhibitors. The 2 open circle lines represent the extreme values found with the various medicaments. See text.

Inhibitors in vitro.—Prostigmine proved to be a potent inhibitor of purifi bovine erythrocyte enzyme but at the same concentration (20 μg/ml) showed effects on the activity of skin homogenates. Propranolol completely inhibit the skin activity at a final concentration of 200 μg/ml. The results are shown in Fig. 1 and Table II.

TABLE II.—*Skin Homogenate Activity at 700 μmol/l β-naphthyl Acetate* 37 °C, *pH* 7·5

Final concentration of inhibitor	% activity
No inhibitor	100
Prostigmine 100 μg/ml	88·8
Propranolol 50 μg/ml	71·3
Propranolol 100 μg/ml	58·3
Propranolol 200 μg/ml	0

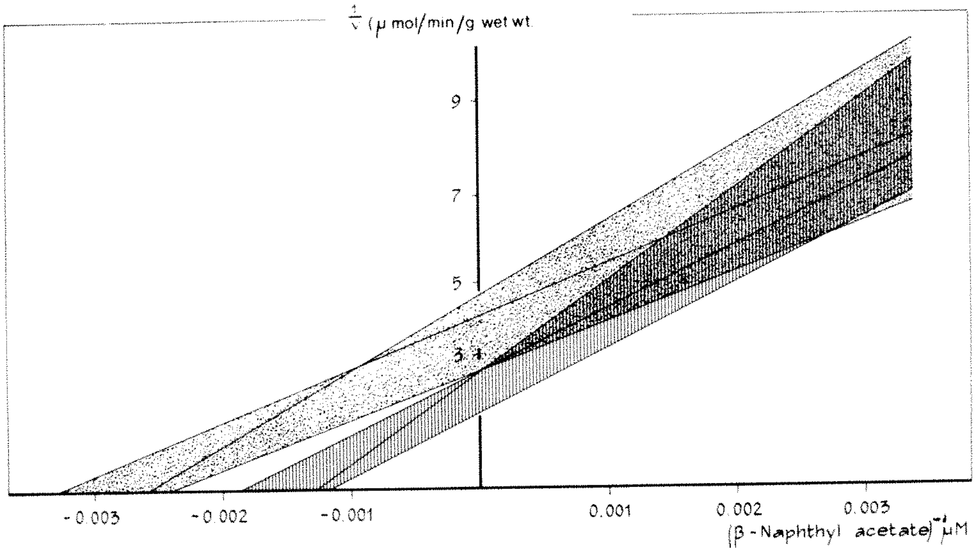

FIG. 2.—Lineweaver and Burke plots of β-naphthyl acetate hydrolysis by skin homogenates made at various times after propranolol (upper range) and saline (lower range) injections.

Propranolol in vivo.—In all cases the propranolol-injected sites showed a reduced $V_{\max}$ and a reduced Km compared to the control sites. The range of values found is shown in Table III. There is no overlap in the Km values between the 2 groups and the $V_{\max}$ values overlap only slightly (Fig. 2). There is no relationship with time of injection from 0 to 24 hours.

TABLE III.—In Vivo *Effect of Propranolol on Enzyme Kinetics of Guinea-pig Flank Skin*

	% of control value	
Time after injection in hours	Km	$V_{\max}$
0	52·7	72·9
0·5	50·0	64·2
1	51·5	54·6
2	73·6	57·7
6	49·8	69·5
24	41·5	82·7

DISCUSSION

The assay method described here is sensitive, rapid, accurate, simple and reliable. It is by definition specific for carboxylic ester hydrolases but not for any one of these enzymes; consequently the kinetic results for any unpurified enzyme system are complex, and inhibitor kinetics even more so. The only difficulty that we have experienced with the technique is with old solutions of β-naphthyl acetate. The solutions are stable for several days, but after this, some breakdown products appear which inhibit the reaction markedly. These breakdown products are not themselves fluorescent and are therefore not noticeable, except that no enzyme reaction occurs. Freshly prepared β-naphthyl acetate solves this problem.

The initial velocity of the reactions was a linear function of enzyme concentration up the level where the reaction began to be inconveniently rapid. The pH optima measurable with the skin enzyme are intrinsically unreliable for this sort of assay, but appeared to fall within the range 7·5–8·5 although much obscured by hydrolysis at low pH values. The β-naphthyl acetate solutions were not significantly hydrolysed at pH 7·5.

The kinetics of β-naphthyl acetate hydrolysis by homogenates prepared from various skin sites varied much more than a series of samples from the same site on different animals. This phenomenon has also been observed in man for the enzyme adenosine 3′5′-cyclic monophosphate phosphodiesterase (Holla *et al.*, 1972), and the general significance of such phenomena has been discussed by Cotton and Mier (1972).

Prostigmine behaved as a competitive inhibitor against the purified bovine erythrocyte acetylcholinesterase, which is in agreement with the literature (Goodman and Gilman, 1970), but the *in vitro* effect on skin was less marked. Propranolol, on the other hand, showed a very powerful inhibition of the skin homogenate activity *in vitro*, but less on the purified erythrocyte preparation. In both cases propranolol appeared to be competitive. The *in vivo* propranolol effect was of the same order as the *in vitro* effect and occurred at the concentration used pharmacologically in sweating studies (Hemels, 1970). The inhibition was obviously complex, since the Km and $V_{\max}$ were both reduced. Such a situation is consistent with uncompetitive inhibition, but the kinetics were not typical of this. It seems more likely that there are a number of carboxylic ester hydrolases in the skin which are variously inhibited by propranolol and that one of these, a high Km species, is inhibited more than the others. Thus the total activity, $V_{\max}$, is reduced and the low Km enzyme "revealed". These points can be elucidated by separating the enzymes and characterizing them.

The significance of these observations for atopy is that atopics are held to behave as though they have propranolol in their skin, at least as far as sweating is concerned. This has previously been related to the Szentivanyi β blockade model (1968). We have now, however, provided direct experimental evidence to support the concept of an abnormality in a carboxylic ester hydrolase, such as a cholinesterase. We are therefore investigating these enzymes in normal and atopic skin and plasma and shall report the findings soon.

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LIPID SYNTHESIS (^{14}C-ACETATE INCORPORATION) IN THE ISOLATED HUMAN SEBACEOUS GLAND, THE APPENDAGE-FREED EPIDERMIS, THE SEBOCYST (STEATOCYSTOMA) AND THE WEN (KERATINOUS CYST OF SKIN)

ROY SUMMERLY AND SHEILA WOODBURY

Summary.—The patterns of incorporation *in vitro* of ^{14}C-acetate into the lipid classes of the isolated human sebaceous gland and overlying appendage-freed epidermis have been compared with those obtained from the sebocyst (steatocystoma) and the wen (keratinous cyst of skin).

The characteristic pattern of incorporation obtained from the wen resembled that of appendage-freed epidermis, thus confirming previous histological and biochemical reports that these cysts are not of sebaceous origin.

Two patterns of incorporation were obtained from the sebocysts; one closely resembled that of the isolated sebaceous gland, and one of the appendage-freed epidermis. These biochemical findings can be directly related to previous histological studies of the sebocyst.

IN an earlier publication (Summerly and Woodbury, 1971) we reported the contrasting patterns of incorporation *in vitro* of ^{14}C-acetate into the lipid classes of isolated human sebaceous glands and of the overlying appendage-freed epidermis.

The common wen (epidermal cyst of scalp; sebaceous cyst) has been variously described as a keratinous cyst of epidermal, pilar or tricholemmal origin (Kligman, 1964; McGavran and Binnington, 1966; Pinkus, 1969); all these authorities agree that the wen is not of sebaceous origin. Nicolaides *et al.* (1968), using conventional T.L.C. methods, demonstrated that the lipid pattern obtained from the contents or the walls of such cysts, whether or not the walls exhibited pale " sebaceous " cells, resembled the pattern of epidermal lipids rather than that of surface lipid from sebaceous gland-rich areas.

The sebocyst (steatocystoma multiplex) has been regarded as a dermoid tumour (Kligman and Kirschbaum, 1964) or a cyst, possibly of sebaceous gland origin, showing sebaceous lobes in its wall (Pinkus, 1969), and its lipid pattern that of " sebum which has not emptied through ducts and follicle onto the surface " (Nicolaides and Wells, 1957).

As the pattern of incorporation *in vitro* of ^{14}C-acetate into the lipid fraction of various skin components reflects lipid metabolism in those tissues, and may be different from the absolute amounts of lipid present, it seemed reasonable to compare the patterns we have obtained from isolated sebaceous glands and appendage-freed epidermis with those from the walls of the sebocyst and the wen.

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MATERIALS AND METHODS

Isolated Sebaceous Glands and Appendage-freed Epidermis

Patterns of incorporation of ^{14}C-acetate into the lipid fraction of isolated sebaceous glands and the overlying appendage-freed epidermis have been obtained from 51 biopsies taken from the upper back of patients suffering from acne vulgaris, or who gave a history of previous acne; patients with a variety of skin disorders; and normal volunteers, using the method described previously (Summerly and Woodbury, 1971) and the results pooled.

Sebocysts

Twenty-four specimens were obtained from the back of a young woman aged 27 who was suffering from steatocystoma multiplex (sebocystomatosis). The cysts were removed under local anaesthesia after making a 4 mm skin incision; the cyst was then punctured and its yellow oily contents expressed, thus permitting the collapsed cyst wall to be removed by gentle traction and dissection (after Kudejko, 1969). The cyst wall was opened out, and any remaining contents were removed by rinsing in 0·9% sodium chloride; it was then placed outer side downwards onto the stainless steel bridge in the culture bottle. In 4 instances half of the cyst wall was taken for routine staining with H. and E.

Wens

Fifteen specimens were obtained from 3 patients (2 females, 1 male) who had multiple lesions on the scalp and neck. Any lesion which showed evidence of a previous inflammatory episode, i.e. could not be dissected cleanly, or which showed partially calcified contents, was excluded from the study. After dissection, the cyst was opened and the soft, sometimes oily or offensive, laminated keratinous contents were gently scraped off; the cyst wall was then placed outer side downwards on the stainless steel bridge in the culture bottle. Routine histology was performed on 3 samples.

Subsequent processing of the cyst specimens.—The culture procedure, extraction of lipids, T.L.C. separation, preparation of autoradiographs and counting methods were as previously described (Summerly and Woodbury, 1971). During extraction, the cyst walls were homogenized in a Potter tissue grinder, using chloroform-methanol 2 : 1 by volume.

RESULTS

(1) *Typical autoradiographic appearances* obtained from isolated sebaceous glands, appendage-freed epidermis, sebocysts and wens appear in Fig. 1 (constructed from a number of autoradiographs to facilitate comparison). Two distinct appearances were obtained from sebocysts, referred to subsequently as patterns A and B. Pattern A (16 of 24) resembled the isolated sebaceous gland, showing dense areas of darkening corresponding to triglycerides, wax esters and squalene. Pattern B (8 of 24) resembled appendage-freed epidermis. When pattern A occurred, the mean total uptake into the cyst wall was 3 times that when pattern B occurred (167×10^3 disintegrations/mg dry weight/100 seconds, as compared with 55×10^3).

The pattern obtained from the wens was constant and distinct, although showing a similarity to appendage-freed epidermis and sebocyst B. It comprised areas of darkening in the polar material at the origin (phospholipid) and in the free sterol (cholesterol) region; activity in the wax ester/sterol ester zone corresponded more closely to the area occupied by the reference compound cholesterol oleate; the area of darkening corresponding to squalene was very much less than in the other 4 tissues studied.

In none of the 4 tissues studied was radioactivity detected at the solvent front, the position occupied in this separation system by saturated hydrocarbons.

(2) *The patterns of incorporation of ^{14}C-acetate in the lipid classes* of the isolated sebaceous glands, sebocyst A, sebocyst B, appendage-freed epidermis and the wen appear in Table I. The overall similarity in pattern between the isolated sebaceous gland and sebocyst A is apparent, the only difference being in the proportion of radioactivity at or close to the origin.

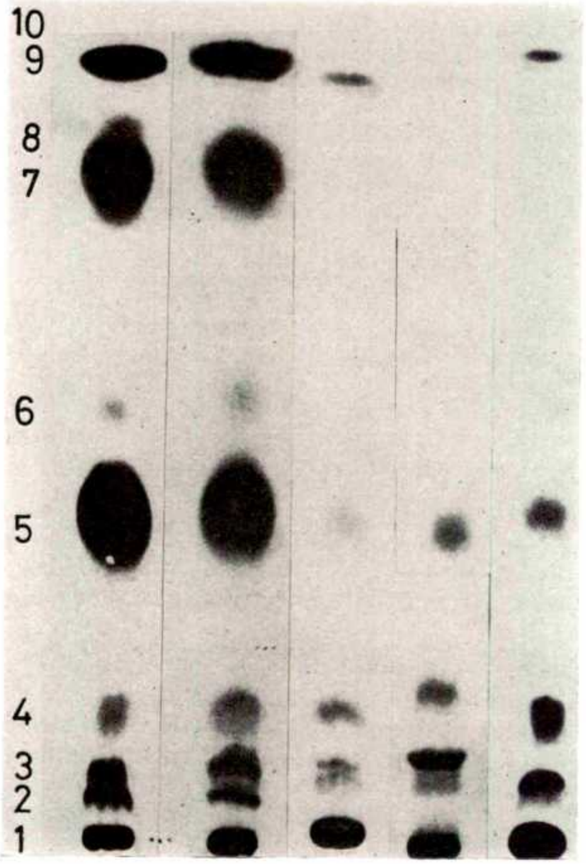

FIG. 1.—Autoradiograph of thin layer chromatogram.

Key: Autoradiograph obtained (reading from left to right) from isolated sebaceous glands; sebocyst pattern A; sebocyst pattern B; wen; appendage-freed epidermis.

Spot 1. Polar lipid material at origin, includes phospholipids
Spot 2. Diglycerides
Spot 3. Free sterol (cholesterol)
Spot 4. Free fatty acids
Spot 5. Triglycerides
Spot 6. Probably polar wax ester (see Summerly and Woodbury, 1971)
Spot 7. Wax esters
Spot 8. Zone containing predominantly sterol esters
Spot 9. Squalene
Spot 10. Solvent front

TABLE I.—*Patterns of Incorporation of ^{14}C-acetate into the Lipid Fraction of Isolated Sebaceous Glands, Appendage-freed Epidermis, Sebocysts and Wens*

(Expressed as % of total incorporation present in each spot)

Tissue	Origin*	Diglyceride	Free sterol	Free fatty acid	Triglyceride	Spot 6	Wax esters†	Squalene
Isolated sebaceous glands (51)	7·8±0·2	1·8±0·1	1·5±0·2	2·0±0·1	45·5±0·7	1·8±0·8	17·0±0·7	21·9±0·8
Sebocyst pattern A (16)	17·0±1·4	2·9±0·4	2·8±0·4	3·7±0·4	40·6±1·4	1·2±0·1	12·3±0·6	19·7±1·6
Sebocyst pattern B (8)	45·2±3·7	11·0±1·3	8·9±2·0	9·4±1·4	14·9±2·0	0·9±0·1	4·3±1·0	5·5±0·8
Wens (15)	38·1±1·8	9·5±1·0	28·6±3·0	10·7±0·9	7·9±1·5	1·7±0·3	1·9±0·5	1·5±0·3
Appendage-freed epidermis (51)	41·2±1·3	8·4±0·5	9·8±0·8	14·5±2·0	14·5±1·0	1·3±0·1	4·6±0·5	6·2±0·5

* = Polar material including phospholipid.

† = Includes sterol esters.

Results are expressed as means ± SEM, with the number of observations in brackets.

The patterns obtained from appendage-freed epidermis and sebocyst B are virtually identical.

The pattern of incorporation obtained from the wens, although bearing an overall similarity to that of appendage-freed epidermis and sebocyst B in that, in all these 3 tissues, more than two-thirds of the radioactivity was present in the phospholipid → free sterol area, showed certain pronounced differences, viz. an increased percentage incorporation into the free sterol band (cholesterol) and a significant reduction in the incorporation into the squalene and wax ester spots.

(3) *The distribution of the incorporated radioactivity in the* 3 *main classes of sebaceous gland lipid in the 5 tissues studied* is shown in Table II. In the appendage-freed epidermis, sebocyst A and sebocyst B, the distribution was the same as that found in the isolated sebaceous gland. In the wen, however, there was a highly significant reduction in the percentage of radioactivity appearing in squalene.

DISCUSSION

The outcome of our investigation, which measures one facet only of lipid synthesis as distinct from an analysis of the total lipid present, is reassuring in that our results correlate well with a previously reported analysis of the lipid pattern of the wen (Nicolaides *et al.*, 1968), and the interpretation of our findings in relation to the sebocyst lies in the detailed histological study of this tissue by Kligman and Kirschbaum (1964).

The pattern of incorporation of ^{14}C-acetate obtained in the wen, viz. substantial labelling of the polar material (phospholipid) and free sterol; some labelling in the free fatty acids and triglycerides; and the labelling in the wax ester/sterol ester zone corresponding largely with the position of the reference compound cholesterol oleate, reflects precisely the lipid pattern of the wen as reported by Nicolaides *et al.* (1968). A small proportion of the total uptake (1·5%) occurred into squalene; Nicolaides *et al.* failed to detect squalene from up to 1200 μg of lipid extracted from the wen. The overall pattern resembles closely that which we have obtained from appendage-freed epidermis, with certain exceptions. In our previous report (Summerly and Woodbury, 1971) we concluded that, as the distribution of radioactivity between the 3 main classes of lipid found in the isolated sebaceous gland was precisely the same in those lipids in the epidermal sample, it seemed likely that the epidermis had been contaminated with sebaceous gland contents during the dissection procedure. This constancy of distribution was not maintained in the epithelium of the wen, the reduction in the proportion of squalene—one

TABLE II.—*Distribution of Incorporation in the* 3 *Main Classes of Sebaceous Gland Lipid in the Isolated Sebaceous Glands, Appendage-freed Epidermis, Sebocysts and Wens*

(Expressed as a % of the total incorporation into the 3 lipid classes)

Tissue	Triglycerides	Wax esters*	Squalene
Isolated sebaceous glands (51)	52·8	21·7	25·4
Appendage-freed epidermis (51)	54·5	22·2	23·3
Sebocyst pattern A (16)	55·0	18·3	26·7
Sebocyst pattern B (8)	58·2	20·3	21·5
Wens (15)	60·8	27·7	11·5

Results are expressed as means, with the number of observations in brackets.
* = Includes sterol esters.

of the most characteristic sebaceous gland lipids—being particularly striking (Table II).

Sebocyst pattern B corresponds even more closely to the pattern of ^{14}C-acetate incorporation into the appendage-freed epidermis, and the " sebaceous " distribution of the 3 lipid classes (Table II) suggests some small contribution from sebaceous elements in the cyst. Sebocyst pattern A closely reflects the pattern of incorporation in the isolated sebaceous gland, indicating that the major contribution to the cyst lipid is derived from functioning sebaceous lobules—the activity in the phospholipid and free sterol areas perhaps reflects the contribution of the keratinizing portions of the cyst lining. Kligman and Kirschbaum (1964) have demonstrated by serial sections that small (or young) sebocysts show mainly a keratinizing lining, with only small intraepidermal nests of cells showing sebaceous differentiation. This anatomical situation would account for pattern B—a keratinizing cyst with a small sebaceous contribution. In Kligman and Kirschbaum's study, large (or mature) sebocysts showed well-developed sebaceous lobules, some in branching outpouchings from the main cyst cavity, and a mainly non-keratinizing cyst lining. This type of cyst would produce pattern A—the contribution from the cyst lining being represented by more labelling in the polar material, free fatty and free sterol zones than is seen in the isolated sebaceous gland.

The failure to demonstrate radioactivity at the solvent front, the position occupied in this separation system by saturated hydrocarbons, in the 5 tissues studied confirms that, when such compounds are detected in analyses of surface lipid, they are contaminants derived from outside sources.

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Br. J. Derm. (1972) **86**, 620.

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IS SULPHUR HELPFUL OR HARMFUL IN ACNE VULGARIS?

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Summary.—Suspensions and creams containing elemental sulphur were shown to be capable of inducing comedones in the rabbit ear and on human backs. Sulphur compounds, sulphides, sulphites, thiosulphates and thiols were inactive in this respect. Even though sulphur was not strongly comedogenic, the thesis is presented that its daily application in the treatment of acne vulgaris might perpetuate rather than ameliorate the disease. It is argued that sulphur has a dual paradoxical role in acne: it hastens the resolution of visible papulo-pustules while insidiously promoting the formation of new comedones from which inflammatory lesions spring, thus establishing a vicious cycle.

MEDICAL history abounds in instances of favoured treatments which were subsequently denounced as ineffective or, worse still, harmful. One likes to think that such happenings occurred in the pre-scientific era of medicine. However, a glance at dermatological treatises up to at least World War II reveals a stunning fascination for toxic chemicals whose worth was blithely accepted without material evidence. The popularity of heavy metals, arsenic, mercury and bismuth left a trail of toxicity which one forbears to review.

Because it is so stubborn and often so serious, acne has been attacked by every conceivable chemical and contrivance. According to the record, practically no treatment has been tried which has been found ineffective; placebos are frequently beneficial in more than 50% of cases (Fry and Ramsey, 1966; Crounse, 1965). One interpretation of such obvious therapeutic bias is that the doctor's imposing presence powerfully counteracts the disease process. Another possibility is that the afflicted adolescent adversely affects the physician's judgment. When the same unhappy patient must be seen for years until spontaneous surcease, the suspicion that one is not greatly helping weakens scientific scepticism. The fact is that acne is a pleomorphic, mercurial shifty disease. Criteria for appraisal of its response to treatment are, to put it quietly, inadequate. Error is constantly at the elbow.

To be obliged to use ineffective drugs is regrettable; it is quite another order of mischief to endorse those which perpetuate the disease process. We propose to argue in this paper that elemental sulphur, a time-honoured remedy in acne, is probably more harmful than helpful. According to traditional dogma, sulphur has multifarious properties which contribute to its efficacy; it is keratolytic,

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anti-bacterial and anti-oxidant. Sulphur is present in a great variety of topical formulations—lotions, cakes, creams, sticks—generally in concentrations of 2–5%, occasionally higher. A large proportion of all anti-acne proprietaries contain sulphur, usually in combination with other agents, of which resorcinol is a typical example. We are pointing an accusatory finger at the precipitate of sulphur, the powdered form of the element. This is the form which enjoys by far the greatest popularity. Our thesis is that elemental sulphur is comedogenic and hence tends to perpetuate the disease by inciting the initiating lesion.

METHODS

Rabbit Assay

The procedure for assaying comedogenicity in the external ear canal of the rabbit has been described previously (Kligman and Katz, 1968). The material was applied to one ear of 2 to 3 rabbits once daily, 5 days a week, for 2 weeks. The opposite control ear received no treatment. An ellipse of tissue was excised on the 14th day, formalin fixed, and sectioned downward horizontally from the surface. Comedogenicity was assessed on a 4-point scale: 0 = not different from the control (untreated ears occasionally exhibit mild hyperkeratosis); 1 = slight distension of follicular lumen and sebaceous ducts with keratinized scales; 2 = moderate accumulation of horny material; 3 = strong hyperkeratosis. It is important to appreciate that the scoring system is relative. A rating of 3 is the maximal response for materials of this class. Potent acneigenic substances such as chloronaphthalenes and tars induce massive horny impactions which might be scored as 10 on the same comparative scale.

Human Assay

We have detailed previously the difficulties of inducing comedones in humans; even with potent acneigens the response is sluggish (Shelley and Kligman, 1957). Individual susceptibility varies tremendously. For weak acneigens, such as pomades and certain cosmetics, 6 weeks of continuous occlusive exposure is the minimum time to secure merely histological evidence (Plewig *et al.*, 1970; Kligman and Mills, 1972).

The subjects were adult male prisoner volunteers. We selected those who had severe adolescent acne earlier, for we have repeatedly found such subjects to be more susceptible to acne venenata. The test area, the upper back, was free of disease. The materials were applied liberally to a 5 cm patch of non-woven cloth (Webril, Curity) which was held in place under an occlusive dressing of Saran Wrap and impermeable plastic tape (3M Blenderm). A control site was covered with a similar, non-medicated patch. A fresh application was made every other day. The patch stayed in place continuously for 6 weeks. The tissue was excised, formalin fixed, usually sectioned horizontally and stained with H. and E. Again, a 4-point scale was utilized.

Test Materials

The agents assayed were either proprietary products containing elemental sulphur or compounds with sulphur in chemical combination. The preparations containing precipitated sulphur were arbitrarily selected so as to be representative of the varieties on the market. Some had additional " peeling " ingredients such as resorcinol, salicylic acid, etc.

RESULTS

Rabbit Assay

The comedogenic potency of various sulphur-containing anti-acne medications is shown in Table I. All those which contained elemental sulphur were moderately

TABLE I.—*Rabbit Ear Comedogenicity of Formulations Containing Sulphur and Sulphur Compounds*

Agent	Other peeling ingredients	Form and % of sulphur	Score		
Sulforcin lotion	Resorcinol monostearate 3%	5% S	2	3	3
Resulin lotion	Resorcinol 4%	8% S	3	3	—
Acnomel cream	Resorcinol 2%	8% S	3	3	3
Clearasil	Resorcinol 2%	8% S	3	3	—
Liquimat lotion	—	5% S	3	3	2
Acnederm lotion	Resorcinol 1·25%	5% S	2	3	—
Fostril	—	2% S	2	1	1
Vleminck's solution (5% aqueous)	—	Calcium polysulphide and thiosulphate	0	0	0
Lotio alba	—	4% Zinc sulphate 4% Sulphurated potash	0	0	0
Komed lotion	Resorcinol 1% Salicylic acid 1%	2% Sod. thiosulphate	0	0	—
Selsun suspension (10% aqueous suspension)	—	2·5% Selenium sulphide	0	0	—
5% Sulphur in Carbowax, U.S.P.	—	—	2	1	1
5% sulphur in Hydrophilic Ointment, U.S.P.	—	—	1	2	1

comedogenic, commonly achieving scores of 3 (Fig. 1). The presence of other " active " components did not prevent this outcome. Certain ones, for example Acnomel®, were fairly irritating grossly and called forth a considerable inflammatory infiltrate even when lightly applied. This did not prevent comedo formation.

Neither of that venerable pair of sulphide melanges, Lotio alba and Vleminck's solution, induced comedones. A thiosulphate-containing proprietary (Komed) was inactive. Though selenium sulphide is not recommended for acne, it too was tested and found to be inert.

Five per cent sulphur in an oil in water emulsion cream (Hydrophilic Ointment, U.S.P.) and in a water soluble polyethylene glycol base (Carbowax, U.S.P.) was less active than the corresponding concentrations in suspension or shake lotions. The effect of concentration was appraised by suspending sulphur in 0·25% Triton X-100, a non-ionic surfactant, in the following percentages: 5·0, 2·5, 1·0, 0·5, 0·25 and 0·1%. The response was strictly dose dependent, the 0·5% concentration being just about threshold. Five per cent was rated 3, 2·5% was 2 or 3 and 1·0% either 1 or 2.

TABLE II.—*Comedogenicity of Sulphur Compounds*

Compound	Rating	
Sodium sulphide (20% aqueous)	0	0
Thiourea (sat. aqueous)	0	0
Ammonium thioglycollate (6% aqueous)	0	0
Sodium bisulphite (5% aqueous)	0	0
Thioacetic acid (5% aqueous)	0	0
Thiosalicylic acid (5% aqueous)	0	0
N-Acetylcysteine (20% gel)	0	0
Cysteiamine hydrate (10% gel)	0	0
Thioacetamide (5% aqueous)	0	0

None of 10 compounds of sulphur exhibited comedogenic capacity (Table II). These included the following radicals: sulphide, sulphite, thiosulphate and thiols.

Human Assay

Elemental sulphur was evaluated as a 5% and 2·5% suspension in 0·25% Triton X-100, as Acnomel (diluted to half strength because of excessive local irritation) and sodium thiosulphate, 5% aqueous. Controls were 0·25% Triton X-100 and Hydrophilic Ointment, U.S.P. A specimen of normal untreated skin was also removed from each subject. This was necessary because a variable amount of hyperkeratosis may occur in some follicles of patients with past acne. A maximum of 4 formulations was tested on one subject.

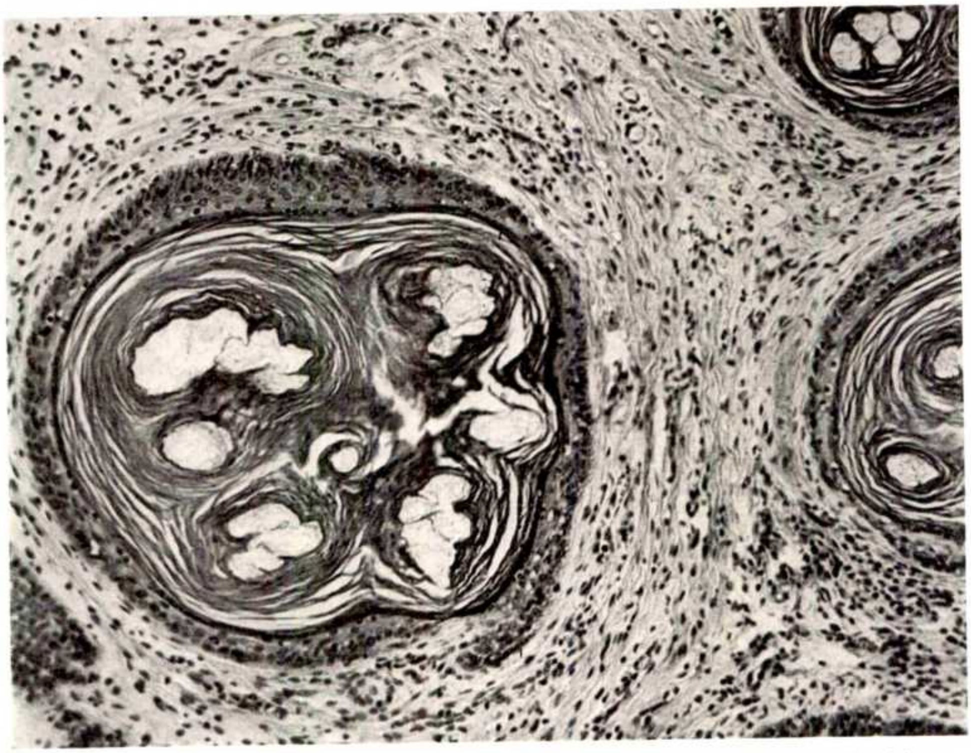


FIG. 1.—Great hyperkeratosis of the sebaceous ducts induced by 10 applications of 5% sulphur lotion to the rabbit external ear canal. Horizontal section. H. and E. × 135.

At 6 weeks there was little to see grossly, except some follicular accentuation and an occasional papulo-pustule. During the first half of the exposure period, 5% S suspension and half-strength Acnomel induced varying degrees of irritation. Histological evidence of incipient comedo formation was consistently observed with all the preparations containing precipitated sulphur (Table III). Sodium thiosulphate was inactive, as were Hydrophilic Ointment and Triton X-100.

The picture resembled early comedo formation in acne vulgaris (Figs. 2, 3). Masses of horny material had begun to accumulate well down in the follicles. Occasional comedones were inflamed, with small accumulations of granulocytes in and around the follicle. Sometimes there was a chronic, perivenular infiltration

TABLE III.—*Comedogenicity in Humans*

Agent	Score					
	1	2	3	4	5	6
5 % Sulphur suspension	2	3	1	2	2	—
2·5 % Sulphur suspension	0	2	1	—	—	—
50 % Acnomel®	1	3	2	2	—	—
5 % Sodium thiosulphate	0	0	—	—	—	—
Hydrophilic Ointment	0	0	0	0	1	0
0·25 % Triton X-100	0	0	—	—	—	—

Each score is for a separate subject.

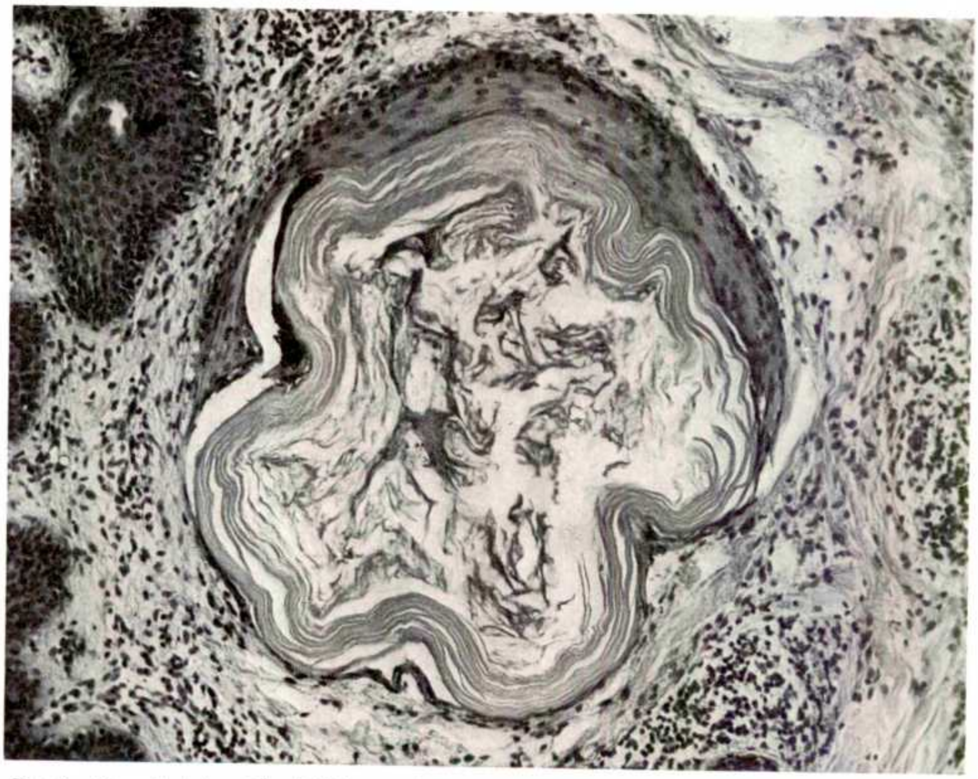


FIG. 2.—Comedo induced by 50 % Acnomel applied under continuous occlusion for 6 weeks to the back. The horny impaction formed, despite clinical irritation, manifested microscopically as a peri-follicular infiltration with mononuclears and erosion of a portion of the epithelial membrane. Horizontal section. H. and E. × 150.

of mononuclears in the upper dermis, especially with Acnomel. These changes reflected the irritation seen clinically.

As in acne vulgaris, some horn-distended follicles showed a great increase in the quantity of gram-positive diphtheroids (*Corynebacterium acnes*) (Fig. 4).

Triton X-100 and Hydrophilic Ointment did not induce hyperkeratosis, except for one mild instance with Hydrophilic Ointment.

DISCUSSION

We have demonstrated that precipitated sulphur, but not sulphur compounds, can induce comedo formation in the rabbit ear and on the human back. Although the evidence is entirely circumstantial, we think it probable that the treatment of acne vulgaris by the daily topical application of sulphur lotions will promote comedones. Acne patients are particularly prone to acne venanata. We will not iterate here the arguments for the credibility of the rabbit model as a predictor of acneigenic potential in man. We have laboured this question elsewhere (Kligman and Mills, 1972). We have described 2 other varieties of acne from external agents which have relied on much the same kind of evidence. The first, pomade acne, occurs very commonly on the foreheads of male negroids who use scalp creams daily (Plewig *et al.*, 1970). The eruption consists mainly of numerous closed comedones which only occasionally become inflammatory. We estimated that it took about a year of daily use for the disorder to become clinically manifest.

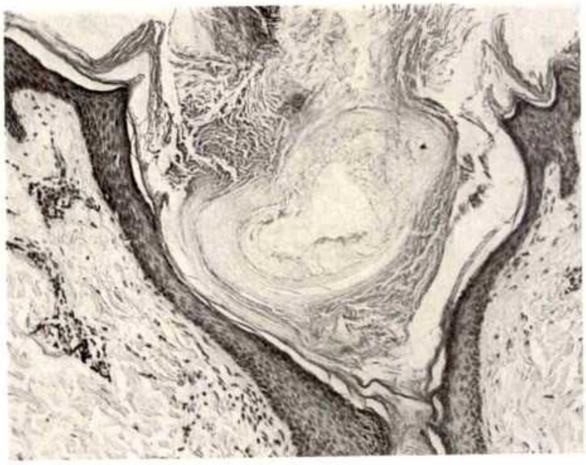


FIG. 3.—Comedo after 6 weeks of 2·5% sulphur suspension in Triton X-100. This small comedo was not definitely visible to the naked eye. H. and E. × 90.

The second variety is acne cosmetica, a low grade eruption which occurs in adult females as a result of the daily use of comedogenic cosmetics (Kligman and Mills, 1972). Here, too, long-term daily use is requisite to create the problem. In relation to the foregoing materials, 5% sulphur lotions have somewhat higher comedogenic potency. We would emphasize that the relation between the use of such ordinary substances and an acneiform eruption is by no means obvious.

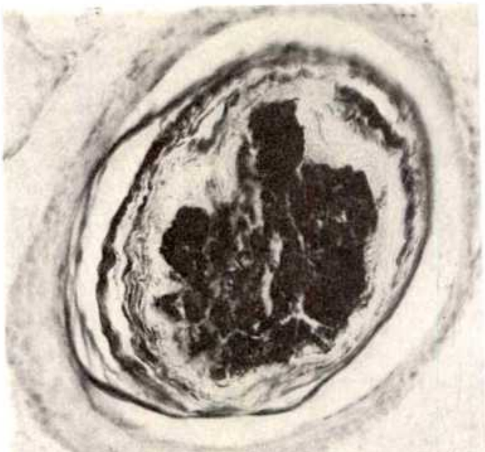


FIG. 4.—Comedo after 6 weeks of 5·0% sulphur suspension in Triton X-100. The control core of black amorphous material is made up of masses of Gram-positive diphtheroids, presumably *Corynebacterium acnes*. Brown–Brenn stain, × 160.

The difficulty of establishing this relationship is intensified in the case of the aggravation of acne vulgaris by sulphur. Is it possible that sulphur has been the mainstay of topical therapy for hundreds of years and may yet have been doing more harm than good? This is a complicated question, for physicians are generally not poor observers.

To resolve this puzzle requires an appreciation of certain peculiarities in the behaviour of the disease. The process begins with the sticking together of horny cells so that an impaction, the comedo, forms and this distends the follicle (Strauss and Kligman, 1960). Initially, the follicular orifice is tiny and the comedo is said to be " closed ". A comedo may be of substantial proportions histologically without being visible clinically. Closed comedones either rupture and become inflammatory papulo-pustules; or, by continued growth, transform into large " open " comedones (blackheads). Attention is called to the fact that closed comedones usually rupture before they become visible clinically. Inflammatory lesions thus seem to emerge independently of visible comedones. None the less, microscopic examination of papules and pustules invariably reveals the incipient horny mass. Long ago physicians found out that " peeling " irritant chemicals would hasten the resolution of papulo-pustules, probably by promoting drainage or by resorption. Peeling is thus desired. Sulphur is one of the classic peeling agents, others being salicylic acid and various phenols. Peeling can also be procured by physical measures: cryotherapy, u.v. irradiation and abrasives. All these clearly help the healing of inflammatory lesions.

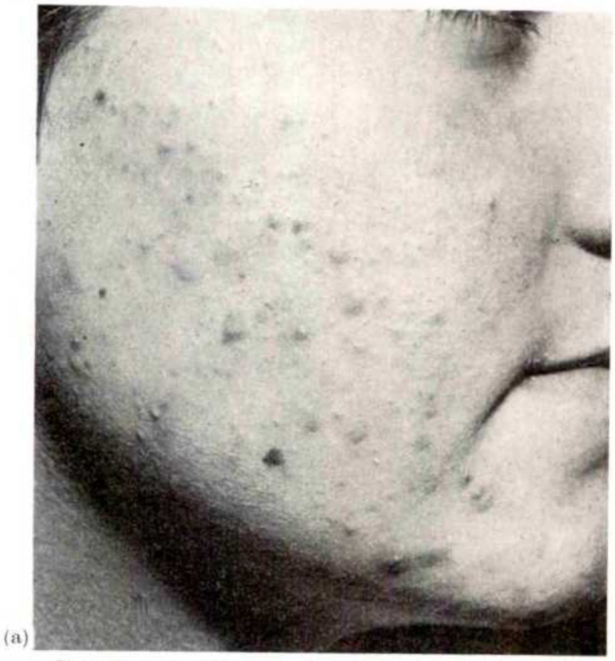
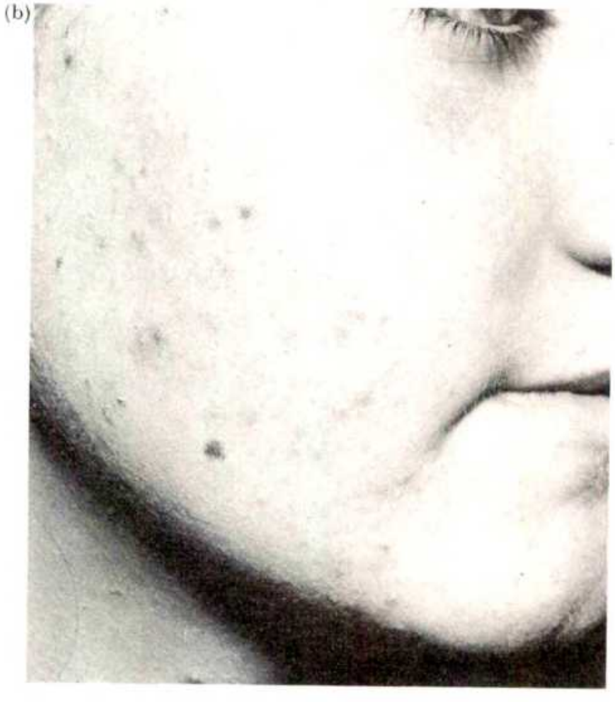


FIG. 5.—(a). Numerous papulo-pustules and comedones before treatment. (b). After 4 weeks of application of a 5% S lotion. The inflammatory lesions, but not the comedones, have diminished.



The role of sulphur is truly subversive, for it potentiates the natural tendency of acne patients to form comedones. This is a rather insidious process, which is not easily detected. Both the patient and the doctor focus more on the irksome vivid papulo-pustules and less on the antecedent comedones; moreover, at an early stage, the latter cannot be seen. The papulo-pustule which might last for a few days or about 2 weeks, depending on infiltration, has a shortened course under the influence of sulphur. At the same time comedo formation is being promoted. The latter is an invisible process which requires many months. Thus a vicious cycle is generated. Sulphur banishes inflammatory lesions and at the same time incites the comedones from which these spring (Fig. 5a, 5b).

The subversive activity of sulphur is hard to expose because it acts on 2 levels: one above the surface in which it is benign, one below in which it is harmful. To be truly efficacious in acne, topical treatments must deter the formation of comedones. In this sense the best treatment is prophylactic. To speed the resolution of inflammatory lesions is comforting, but not very constructive. Like all successful villains, sulphur has flourished so long because it is attractive at first glance. It takes a hard look beneath the surface to spoil the game.

Our observation of masses of *C. acnes* in sulphur-induced human comedones raises serious doubts concerning its reputation as an antibacterial agent. Perhaps the only facet of sulphur which will never be brought into question is its bad smell.

Now that more effective peeling agents are available, for example benzoyl peroxide and retinoic acid, it would seem time for elemental sulphur to make an elemental exit.

Finally, we would cite the quite incidental observations of Van Scott (personal communication) in support of the notion that sulphur is comedogenic. He has utilized the hairless " rhino " mouse as a model to appraise the efficacy of topical anti-acne medications (Van Scott, 1972). It was found that sulphur preparations actually increased the comedo-like follicular impactions which develop spontaneously in this animal.

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TOPICAL TREATMENT WITH BAY b 5097, A NEW BROAD SPECTRUM ANTIMYCOTIC AGENT

TORSTEN FREDRIKSSON

Summary.—The effect on superficial mycoses of BAY b 5097, a tritylimidazole derivative, applied topically in a 1% solution, was evaluated in a randomized double-blind study in 54 patients. It was significantly better than a placebo in diseases produced both by *Candida albicans* and by Trichophyton species. After 3 weeks of treatment, clinical healing was recorded in 57% of the cases and marked improvement in an additional 33%. No side effects were noted.

BAY b 5097, bis-phenyl-(2-chlorphenyl)-1-imidazolylmethane or chlotrimazol (Fig. 1), is a new antimycotic agent with broad spectrum properties. This tritylimidazole derivative, which is insoluble in water but readily soluble in lipid solvents, has fungistatic activity *in vitro* towards various species of Candida, Trichophyton, Microsporum, and *Epidermophyton floccusum* and also towards many fungi causing deep mycoses. It has already been used successfully for the oral treatment of mainly generalized candidiasis (Oberste-Lehn *et al.*, 1969, 1970; Marget and Adam, 1969, 1971; Plempel *et al.*, 1969). It therefore seemed justifiable to try to evaluate the efficacy of BAY b 5097 used topically in the treatment of superficial dermatomycosis.

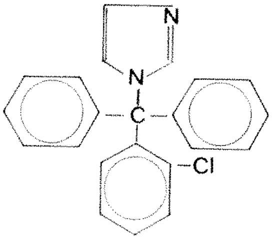

Fig. 1.—Structural formula of Bay b 5097 (diphenyl-(2-chlorphenyl)-1-imidazolylmethane).

MATERIALS AND METHODS

The study was designed as a randomized, double-blind trial in which approximately half of the patients were started on active material and the others on a placebo. The active material was a 1% solution in polyethylene glycol 400; and polyethylene glycol 400 served as the placebo. Both materials were of identical appearance and labelling, and were marked with code numbers by the manufacturer.

The original material consisted of 80 outpatients with typical dermatomycotic conditions (candidiasis or tinea). Direct microscopical demonstration of fungi was not regarded as sufficient evidence of fungal disease, and cases were included in the study only where fungal cultures were also positive. On the first visit, specimens for culture

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were taken, and DTM-medium was used (Taplin *et al.*, 1969). In no case was there any clinical doubt that the cultured pathogen was the predominant cause of the disease. The patients received either the active material or the placebo in a randomized fashion and were instructed to apply the solution twice daily, using no form of dressing. After 3 weeks of treatment the patients were seen again, further specimens for culture were taken, and the condition of the disease was judged according to the following scheme: unchanged or worse, slight improvement, marked improvement, or healed. Then the code was broken, and those patients who had been started on the placebo were switched to active treatment. These patients were seen again after 3 weeks, when the effect of the treatment was evaluated and new specimens for culture were taken. Of the original 80 patients 20 were excluded due to the fact that the first or, in the case of placebo treatment, the second culture did not show any growth, in spite of the typical clinical features of mycotic disease. Six patients who were started on the placebo were also excluded instead of being switched to the active material, since they preferred conventional therapy.

The final material thus consisted of 54 patients. Of these 31 were started on the active material (14 in the candidiasis group and 17 in the tinea group) and 23 on the placebo (13 in the candidiasis group and 10 in the tinea group). There were 29 men and 26 women. The age varied between 5 and 84 years, with a mean of 42·1 years. The duration of disease varied between 2 weeks and 20 years, with a mean of 22·7 months. The duration of disease was particularly long in the candidiasis group. In 27 of the patients the causative organism was *Candida albicans* and in the remaining 27 various dermatophytes (in 20, *Trichophyton rubrum*; in 5, *T. mentagrophytes*, in one a Trichophyton species belonging to the crateriform group, and in one *Epidermophyton flocosum*). The clinical diagnoses are summarized in Table I.

RESULTS

Of the 23 patients who were started on the placebo, 19 were unchanged or worse after the first 3 weeks of treatment, while slight improvement was noted in the remaining 4 (one in the candidiasis group and 3 in the tinea group). These 4 patients healed completely after treatment with the active material. The second fungal culture was positive in all patients who were started on the placebo and included in the trial, and showed growth of the same organism as in the first culture. After active treatment the fungal culture was negative in all 54 patients.

The results of active treatment are summarized in Tables I, II. After 3 weeks of treatment, 57% of the patients had no clinical evidence of disease; 44% of those with candidiasis and 70% of those with tinea. This difference is not

TABLE I.—*Clinical Diagnoses and Results of* 3 *Weeks' Treatment of* 54 *Patients with* 1% *BAY b* 5097 *In Propylene Glycol*

Clinical diagnoses	Unchanged or worse	Slight improvement	Marked improvement	Healed	Total
Candidiasis					
Paronychia	1	—	5	4	10
Inguinal	—	1	3	3	7
Intertrigo	—	2	2	1	5
Cheilitis	—	—	1	2	3
Erosio interdigitalis	—	—	—	2	2
Tinea					
Pedis	—	—	5	12	17
Inguinalis	—	—	—	7	8
Capitis	1	—	—	—	1
Corporis	—	—	1	—	1

TABLE II.—*Overall Results after 3 Weeks' Treatment of 54 Patients with 1% BAY b 5097 in Propylene Glycol*

Fungal disease	Unchanged or worse	Slight improvement	Marked improvement	Healed
Candidiasis	1	3	11	12
Tinea	1	—	7	19

statistically significant (χ^2-analysis), but it was the impression of the investigator that cases with tinea responded more rapidly and better than those with candidiasis. However, the efficacy in the treatment of chronic paronychia was striking, not only in comparison with the placebo but also with earlier conventional therapy, including nystatin.

Of the 33 patients who had received other active therapy on earlier occasions, 25 regarded BAY b 5097 as superior, 7 as equally effective and only one thought it inferior. The patient with candidal paronychia who did not respond to treatment had also a superinfection with *Staphylococcus aureus*. The patient with tinea capitis produced by a Trichophyton species of the crateriform group had a very severe infection that did not respond particularly well on griseofulvin given later. No side effects were observed, neither irritation nor allergic contact dermatitis.

DISCUSSION

The present study indicates that BAY b 5097 in the concentration and vehicle used is effective following topical application in both candidiasis and tinea. This broad spectrum is a unique and valuable property, but the compound's definite place in the treatment of dermatomycosis has yet to be established. Investigations of larger series of cases, comparisons with traditional therapy and studies on recurrence rate are necessary. The possible efficacy in various forms of onychomycosis is also of interest, and our study has been extended to this condition, but results are not yet available. No side effects were noticed, and in 11 patients, where haematology, liver and kidney function could be studied before and after treatment, no abnormal values appeared. Further toxicological data are necessary for obvious reasons, but may certainly be obtained from studies with oral treatment.

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HYPERBARIC OXYGEN THERAPY IN DERMATOLOGY

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Summary.—Successful results are reported with hyperbaric oxygen treatment of 6 dermatological cases in which previous methods of therapy have been unsatisfactory. In one case of advanced generalized scleroderma, regression of the skin contraction was achieved, together with improved mobility of joints and healing of persistent ulcerations. In 2 cases of severe arterisoclerotic leg ulcers healing of the ulcers was achieved without mutilating operations. In one case each of progressive pyoderma gangrenosum, ulcer of Felty's syndrome and fulminant bullous haemorrhagic erysipelas, full healing of the ulcers was also achieved. The nursing periods were undoubtedly greatly shortened, and the hyperbaric treatment could to a large extent be given on an ambulatory basis. No adverse side-effects were observed.

OXYGEN treatment at elevated atmospheric pressure is an effective but hitherto little used method of counteracting local tissue hypoxia due to restricted peripheral circulation. Of dermatological interest is the use of this method for the treatment of poorly healing or persistent ulcers, especially as the suppression not only of anaerobic but also of aerobic infections has been observed (McAllister *et al.*, 1963; Irvin and Smith, 1968). The method has been used for the treatment of leg ulcers of both arteriosclerotic and varicose character (Illingworth, 1962; Bird and Telfer 1965; Slack *et al.*, 1966; Barthèlemy *et al.*, 1967; Mantz and Tempe, 1968; Besznyák *et al.*, 1968; Shaldin, 1969; Barr and Benson, 1970; Bass, 1970; Kidokoro *et al.*, 1970; Lefebvre *et al.*, 1970).

Other than leg ulcers only a few dermatological diseases seem to have been subjected to hyperbaric oxygen treatment. Duluc *et al.* (1968) reported 3 cases of psoriasis. One case of purpura fulminans was reported by Wadell *et al.* (1965) and another was reported by Kuzemko and Loder (1970). Three cases of chronic undermining ulcer of Meleney were reported by Grainger *et al.* (1967). Dowling *et al.* (1967) reported a good effect on Raynaud's phenomenon in 6 cases of scleroderma.

In the present study the use of hyperbaric oxygen (HPO) has been extended to certain pathological conditions in which local circulatory insufficiency is evident and/or the healing process needs an unduly long period of hospitalization.

MATERIALS AND METHODS

Six patients are presented: one suffering from generalized scleroderma, one from pyoderma gangrenosum, one from Felty's syndrome, one from bullous haemorrhagic erysipelas and 2 from arteriosclerotic leg ulcer.

The treatments were all given in a one-man plexiglass pressure chamber (Vickers type CH S/3) at a pressure between normal and 2·5 times normal atmospheric pressure, i.e.

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at 1–2·5 atmospheres absolute pressure (atma). The pressure was increased in 5–6 min, kept constant for 1–1·5 hours and lowered in 3–4 min. A continuous flow of oxygen (300 l/min) passed through the chamber during the treatments to avoid rebreathing of CO_2. As a routine, all patients had the lungs controlled by x-ray and the eardrums and Eustachian tubes checked before the first treatment. Only exceptionally was premedication (100 mg pentobarbital) given.

Case Reports

Case 1.—A.S., a woman aged 33. Diagnosis: generalized scleroderma.

For 3 years the patient had suffered from progressive generalized scleroderma, which had been treated with corticosteroids, immunosuppressive agents and antibiotics without effect. At the start of HPO the patient was seriously disabled, with a pursed mouth, and almost no mobility in finger, wrist and elbow joints. Minor ulcerations over these joints were also present, as well as a palm sized infected ulcer over the right medial malleolus. After 3 weeks of HPO, the scleroderma process was apparently arrested. Both the patient and her relatives spontaneously reported that the skin had become less tense and of a healthier colour, and that joint mobility was increased. After 7 months of treatment a further regression of the scleroderma was seen. Thus there was less stiffness in the joints and an almost normal facial expression. The leg ulcer was healed with autografts from the abdomen. In all 328 HPO treatments were given in 9 months. The good condition still persists 5 months after the end of treatment.

Case 2.—G.K., a man aged 49. Diagnosis: ulcerative colitis and pyoderma gangrenosum.

He had suffered repeated attacks of ulcerative colitis and pyoderma gangrenosum for 7 and 6 years respectively. HPO, 3 years ago, primarily intended to eradicate anaerobic bacteria in the bowel, had been found also to promote healing of the leg ulcer. A new ulcer appeared after 2 years. This time HPO showed no significant effect until the treatment was intensified from 1 hour at 2 atma once a day to 1 hour at 2·5 atma 2–3 times a day. The ulcer healed after 81 treatments.

Case 3.—M.H., a woman aged 54. Diagnosis: rheumatoid arthritis and leg ulcers (Felty's syndrome).

Rheumatoid arthritis, present for 16 years, had been treated with continuous oral steroid therapy for 13 years. In the last 4 years the patient has been repeatedly hospitalized for a total of 22 months with leg ulcers of the type seen in Felty's syndrome. After 6 weeks of HPO the appearance of bright red granulations allowed the ulcers to be covered with split-skin grafts. The patient was discharged fully healed after 3 months and 99 hours of treatment. There has been no recurrence during 10 months of observation.

Case 4.—E.T., a man aged 67. Diagnosis: bullous haemorrhagic erysipelas.

Erysipelas, with multiple haemorrhagic bullae, developed on the left lower leg. Despite adequate penicillin treatment, according to bacterial cultures, 6 large deep ulcers had appeared 1 month after the onset. Within 2 days of the start of HPO, red granulations could be seen among the necrotic tissues. The condition progressively improved and, after 3 months and 130 hours treatment, 5 of 6 ulcers were completely healed. There was no recurrence during 7 months of observation.

Case 5.—S.B., a woman aged 56. Diagnosis: leg ulcer (arteriosclerotic).

A rapidly growing, pretibial ulcer on the left lower leg developed after a slight injury. About 7 weeks later the ulcer almost encircled the leg. Femoral angiography showed the common iliac artery to be totally obliterated 4 cm distal to the bifurcation. Via the collateral circulation contrast filling of the vessels was obtained about 15 cm distal to the obstruction. Despite intense local treatment and systemic chemotherapy, the ulcer grew to a 20–25 cm wide gaiter surrounding the leg. Amputation was recommended repeatedly, but the patient refused. As a last resort, it was decided to start HPO.

After about 1 month it was possible to apply split-skin grafts. HPO was continued. The patient was discharged healed 3·5 months after the start of HPO and she resumed her occupational activities to the full extent 2 months later. One year later the skin is still intact.

Case 6.—E.L., a man aged 67. Diagnosis: leg ulcer (arteriosclerotic).

The right femur was amputated 2 years ago. Six months later a gangrenous, severely painful ulcer on the sole of the left foot appeared and grew to 5 × 6 cm in 2 months. In the femoral artery satisfactory pulsations could be felt, but none in the popliteal, posterior tibial or dorsal arteries of the foot. Oscillometry suggested stenotic lesions. After 6 weeks of HPO (total 69 hours) the ulcer was fully healed. At a follow-up 11 months later there were no signs of recurrence.

DISCUSSION

There is at present no satisfactory therapy for generalized scleroderma. Corticosteroids do not appear to affect the process. The value of immunosuppressive therapy is the subject of much discussion. Low molecular weight dextran has been tried, especially when acrosclerosis is present. The patients have felt a little warmer in the fingers, and ulcerations have healed, but renal complications have occurred. Our patient (Case 1) was in an advanced stage of the disease and was well aware of the poor prognosis when the hyperbaric oxygen treatment was introduced. The physical improvement had a positive psychological effect on the patient as well as on her family, and the increased mobility meant that she needed less help with daily attendance. The treatment admittedly did not eliminate the disease, but led to a distinct regression of the lesions. Even if the patient should need recurring treatment periods in the future, this therapy is well justified despite its cost in time and money. The successful healing of the ulcer on the leg as well as the graft donor site seems to indicate that the hyperbaric treatment has eventually normalized the healing capacity of the diseased skin.

Pyoderma gangrenosum is not uncommon in patients with ulcerative colitis, but often appears also in patients with rheumatoid arthritis. Apart from treatment of the basic disease, the therapy of the skin lesions has been solely symptomatic. In rheumatoid arthritis other types of leg ulcers also occur. One type is seen in Felty's syndrome, in which the ulcers are often multiple, painful, slowly healing and frequently recurring. In the patient with pyoderma gangrenosum (Case 2) and in the patient with an ulcer of Felty's type (Case 3) the hyperbaric treatment led to a marked shortening of the expected hospitalization periods. In the pyoderma gangrenosum case no positive effect was obtained until the pressure had been increased to 2·5 atm and the duration of treatment had been prolonged to 1·5 hours on each occasion. In the patient with an ulcer of Felty's type, the treatment with oxygen had to be continued after the split-skin graft. In accordance with our earlier experiences with hyperbaric oxygenation, the best survival rate for the split-skin coverings was gained with the oxygen pressure in the chamber limited to 1 atm, i.e. pure oxygen at normal atmospheric pressure, during the first few days after application.

The patient with bullous haemorrhagic erysipelas (Case 4), who quickly developed large, deep, purulent necrotic ulcers, would most likely have needed extensive skin grafts, and perhaps even amputation, but for the use of the hyperbaric chamber. In future we intend to start oxygen treatment of such cases of severe, bullous haemorrhagic erysipelas at the earliest possible stage of the disease, hoping to reduce the spread in depth and thus shorten the time of treatment.

Several authors (Slack *et al.*, 1966; Barr and Benson, 1970; Bass, 1970) have reported the favourable effect of hyperbaric oxygen on leg ulcers caused by venous insufficiency, with inhibition of bacterial growth, increased vascularization and improved epithelialization. The value of hyperbaric oxygen on arteriosclerotic leg ulcers, on the other hand, has been less conclusive (Gorman *et al.*, 1965; Fischer, 1969; Bass, 1970). In these cases the patients have as a rule been very old, and the treatment was given as a last resort when the usual means of therapy had been exhausted. Slack *et al.* (1966) mentioned that the limited treatment programme they used, with only one session a day, might be responsible for their poor results. The positive effect in Case 5, in which the patient was treated up to 3 times daily, seems to confirm this hypothesis. The control period of only 12 months is rather short; but, even if the treatment had to be resumed in the future, much has been gained, as the alternative was amputation. The relatively low age of this patient, 56 years, may have been favourable with respect to her ability to develop collateral circulation. The other patient (Case 6), with an ulcer of arterial type, was also saved from an imminent amputation.

The results of treatment in the 6 cases presented show that the hyperbaric oxygen technique may have important therapeutic potential in dermatology. It attacks the problems from a new angle, which definitely leads to shorter periods of treatment and sick leave. Further, it is gratifying to note that ambulatory treatment could be applied to a large extent. No troublesome side-effects have been observed.

A short summary of the physiological background to hyperbaric oxygenation and the practical implications of its use may be helpful for those not familiar with this relatively new form of treatment.

An increase in the partial pressure of the oxygen in the lungs from 100 mm to 1500–2000 mmHg will cause a proportionately greater quantity of oxygen to be taken up by the arterial blood in physically dissolved form. It is thus possible, at least partly, to compensate for a decrease in the blood flow by an increase in the oxygen content of the blood. Further, the increased partial pressure of the oxygen in the blood will increase its capacity to diffuse into the tissues from the vascular bed. A direct local effect of the ambient oxygen on open and often infected wound surfaces is also considered to improve the healing conditions. A persisting effect, probably due to local revascularization, appears to be attainable also in elderly patients when subjected to extended treatment programmes. To achieve a sufficiently high oxygen pressure, the atmospheric pressure around the patient has as a rule to be increased to 1–2 atmospheres gauge pressure (atmg), corresponding to 2–3 atmospheres absolute pressure (atma). The hyperbaric oxygen treatment, therefore, needs special pressure chambers accommodating one or more persons. To avoid oxygen cramp and lung injury, each treatment should not exceed 3 atma and should not last longer than 2 hours. Instead, the exposure should be repeated one or more times per 24 hours. If necessary, the treatment may be extended over several months. There is no risk of decompression sickness, due to nitrogen bubbles appearing in the blood when the pressure is lowered, as only oxygen is breathed. The greatest risk is, rather, oxygen intoxication. The oxygen fits or cramps, however, quickly disappear on lowering the pressure, and the treatment can be continued on the following day. The pressure equilibration in the middle ear during the raising of the pressure, as a rule presents no problems. It should be mentioned, however, that, during very extended treatment programmes at higher pressure levels, a cataract-like clouding of the lens in arteriosclerotic patients has been seen (Pallin and Barr, 1972). The indications for excessive hyperbaric oxygen treatment should thus be relative and the advantages of, for example, a saved limb should be weighed against the eventuality of a future cataract operation.

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Case Reports

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GRANULOMA ANNULARE IN IDENTICAL TWINS

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Summary.—Granuloma annulare in proven identical twins is described. Both patients were synalbumin-positive.

We have found granuloma annulare in proven identical twins and felt that this should be recorded since, in the only previous report (Schiff and Cohen, 1953), genetic identity was assumed but not proved.

Clinical Findings

Case 1.—Angela G. (birth weight 2·36 kg), a school teacher aged 23, presented with papular lesions on several fingers of both hands, some arranged in an annular fashion. Her family history revealed that her twin sister had similar lesions; there was no family history of diabetes or of organ-specific auto-immune disease.

Case 2.—Ann G. (birth weight 1·97 kg), also a school teacher, was examined and found to have similar papular lesions on the right index finger. Both twins were apparently healthy and gave no history of previous disease.

Investigations

Genetic studies.—Using 7 single gene systems, *O*, *CCDee*, *MsNs*, Fy^{a+b-}, Jk^{a+}, $Le^{a-}/Se+$, and 2 dermatoglyphic characters summed at angles 87° and 86°, total ridge count 13 and 20, the probability that these sisters are identical twins was 98·7%.

Synalbumin antagonism.—Serum from both patients was kindly examined by Vallance-Owen (Vallance-Owen *et al.*, 1955), who demonstrated significant insulin antagonism associated with the albumin fraction (synalbumin-positive).

Histology.—The histology of a papule from each patient was entirely consistent with granuloma annulare, showing collagen degeneration and associated histiocytic reaction. Necrobiosis was limited and occurred in discrete foci.

Glucose tolerance tests.—Normal in both patients, with no renal glycosuria.

The prednisone glycosuria test (Joplin *et al.*, 1961).—Normal in Ann G., with a glucose excretion per hour of 4 mg, but grossly abnormal in Angela G. who excreted 125 mg glucose per hour.

These tests were performed twice on each patient, with similar findings respectively, on each occasion. A full blood count was normal, serum lipids normal, and the urine was free from reducing substances.

DISCUSSION

Vallance-Owen (1966) believes that excessive synalbumin antagonism is a biochemical marker of the diabetic constitution which may ante-date carbo-

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hydrate intolerance. In association with Dr Grace Allen he found that, of 14 patients over the age of 15 years with granuloma annulare, 10 were synalbumin-positive (Vallance-Owen, 1969).

The fact that our twin patients were synalbumin-positive is in accord with this finding.

We are most grateful to Dr D. F. Roberts, Laboratory of Human Genetics, University of Newcastle upon Tyne, for carrying out the genetic studies and to Professor J. Vallance-Owen, Department of Medicine, Queen's University of Belfast, for carrying out the synalbumin estimations.

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RECURRENT ECZEMA HERPETICUM ASSOCIATED WITH ICHTHYOSIS VULGARIS

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Summary.—Two adult patients with both dominant ichthyosis vulgaris and a past history of atopic eczema, who developed recurrent attacks of eczema herpeticum, are described.

Kaposi's varicelliform eruption (KVE) results from infection with *Herpes simplex* or vaccinia virus in patients with certain skin diseases. The condition usually arises from primary infection with the particular virus and causes constitutional upset. Most cases occur in the presence of atopic eczema and in children, but atopic subjects without active skin disease are also susceptible, and patients with pemphigus foliaceus (Silverstein and Burnett, 1967), Darier's disease (Gerstein and Shelley, 1960; Loeffel and Meyer, 1970; Izumi and Goldschmidt, 1970) and ichthyosiform erythroderma (Fitzgerald and Booker, 1951) are sometimes affected.

KVE is preferably called eczema herpeticum when *Herpes simplex* is the virus concerned. Recurrent attacks of eczema herpeticum are recognized, but are rare, and tend to be milder than the initial attack.

This paper describes 2 patients with eczema herpeticum in whom recurrent attacks occurred. In the first patient a recurrent attack was more severe than the earlier ones; in the second patient each successive attack was less severe.

CASE REPORTS

Case 1

History.—A dental surgeon aged 26 was admitted to hospital as an emergency. He gave a history of dry skin since birth and of eczema since early infancy; he had experienced little discomfort from the eczema since the age of 15 years.

Nine months before admission he had had a vesicular and pustular eruption in the antecubital and popliteal fossae, which cleared spontaneously, and again 6 weeks before admission he suffered from a vesicular and pustular eruption over the upper limbs, which cleared rapidly.

One week before admission and unassociated with any activity of his flexural eczema, painful vesicles appeared over the backs of his hands, rapidly spreading to forearms, chest, neck and face; his face had also become swollen as the vesicles appeared. Three days later he began oral prednisolone 40 mg daily, but there was no improvement. His wife had had a " cold sore " on a lip one week before he developed his eruption. When admitted to hospital he complained of painful hands, anorexia and constipation, and he had noted his urine to be dark in colour.

On examination he looked unwell, but was afebrile. His ichthyotic skin showed a vesicular and pustular eruption on the face, neck, forearms and hands (Fig. 1). Some lesions were impetiginised, and many groups of vesicles showed surrounding erythema.

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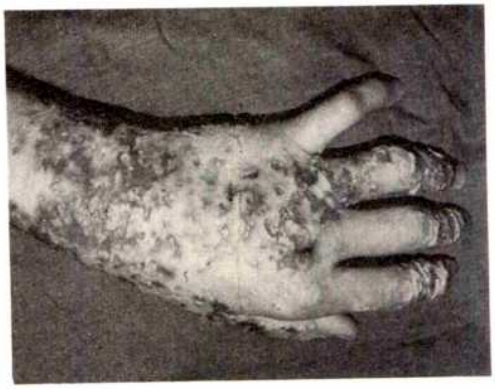
It was noticeable that much of the eruption was on exposed areas and that areas unaffected by eczema were involved. There was cervical, axillary and inguinal lymphadenopathy. Neither liver nor spleen was palpable.

Investigations.—On admission: WBC 5000/mm^{-3} (N 56; L 32; E 1; M 11) and ESR 40 mm in 1 hour (Westergren). Of the liver function tests, serum bilirubin and alkaline phosphatase were normal, but SGOT was 42 units and HBD 286 units. Gel test for Australia antigen was negative. Blood culture was sterile. Swab from a hand grew *Staphylococcus aureus* and a β-haemolytic streptococcus belonging to Lancefield's Group A. *Herpes simplex* was cultured from a vesicle swab. Urine showed an excess of urobilinogen. Chest x-ray was normal. Complement fixation test showed antibodies to *simplex* virus 1 in 128. Convalescent serum taken 2 weeks after the first specimen still showed an antibody titre of 1 in 128, confirming that the *Herpes simplex* infection was a recurrent rather than a primary one. The SGOT and HBD had fallen to normal by the eleventh day after admission.

Treatment and progress.—Initially 5% aluminium acetate soaks were applied to affected areas, followed by hydrocortisone with oxytetracycline ointment; 5% idoxuridine in dimethyl sulphoxide was applied to intact vesicles, and oral erythromycin was given. A single dose of human hyperimmune γ-globulin, 750 mg intramuscularly, was administered 2 days after admission, and the oral prednisolone begun before admission was reduced gradually and discontinued after one week of hospital treatment. He made a slow but uneventful recovery.



Case 2

History.—A Post Office counter clerk aged 20 suffered from atopic eczema from infancy until 15 years old. Although he had had no active eczema since then, longstanding dryness of the skin persisted. There was a strong family history of atopic disorders. The patient gave no past history of *Herpes simplex*.

Two weeks before the acute illness, his girl friend had suffered from a " cold sore " on her lip, and one week later he suffered from an influenzal type of illness with fever, lassitude and sore throat. After 4 days with these symptoms, groups of vesicles and pustules developed on his face and around his neck, causing considerable swelling.

On examination there was a pyrexia of 38·8°C. The patient appeared flushed, and there were enlarged, tender lymphatic glands in the submandibular region and neck. The skin of the face and neck was erythematous and extensively involved in a papulovesicular and pustular eruption with crusted exudate, which in places formed confluent sheets. The face and neck were markedly oedematous. Elsewhere the skin was ichthyotic, but without evidence of active eczema. The rest of the physical examination was normal.

Investigations.—Swabs from the skin lesions grew *Herpes simplex* virus. No pathogenic bacteria were demonstrated. Total WBC 5700/mm^{-3} (N 60; L 31; M 9) and 2 Turk cells were noted in the peripheral blood film. Chest x-ray was normal.

Treatment and progress.—Systemic ampicillin and 2 doses of hyperimmune human γ-globulin (250 mg and 750 mg) were administered, and the skin bathed with normal saline before the application of 3% tetracycline ointment. Resolution of the pyrexia, facial swelling and pustular eruption occurred rapidly, and in one week the patient was fit to be discharged from hospital.

One year after the first episode the patient attended again with a similar vesicular and pustular eruption around his neck. There was little systemic upset, and the severity of the attack was much less than the first. No pyrexia occurred, and the eruption cleared in less than 2 weeks, with symptomatic therapy only. Again *Herpes simplex* was isolated from the lesions. A complement fixation test performed on a serum sample taken during the acute phase showed an antibody titre of 1 in 10, and convalescent serum taken 3 weeks later showed no change in titre. In the acute phase the serum total protein level was 7·6 g/100 ml, with a prominent β-globulin band on electrophoresis. The globulin levels were IgG 1900; IgA 225; IgM 50 mg/100 ml (normal: IgG 800–1500; IgA 140–400; IgM 50–200) and at the convalescent stage had not altered significantly.

Ten months after the second episode, a further attack of well-circumscribed, erythematous, vesiculo-purulent lesions occurred around the left eye and gradually spread in a localized area. The severity of this third attack was much less than that of the previous ones and the lesions cleared quickly with 5% idoxuridine in dimethyl sulphoxide. On this third occasion *Herpes simplex* virus was again grown from the lesions.

DISCUSSION

Eczema herpeticum is usually a manifestation of primary infection with *Herpes* virus *hominis* and is therefore most common in infancy. Recurrences over a period of years are seen occasionally (Boake *et al.*, 1951; Mailman *et al.*, 1964). Mailman *et al.* (1964) described a boy with recurrent eczema herpeticum who had an attack when his skin was clear of active atopic eczema. The patients described in our paper presented to us in such attacks. The first patient had had at least one similar but probably 2 milder attacks previously. The second patient was seen in all 3 attacks, and virus was grown from lesions each time.

Herpes simplex hepatitis in an adult has been reported (Flewett *et al.*, 1969), but is a rare occurrence. It seems likely that the first patient had a transient abnormality of liver function as part of his systemic upset.

Although a personal or family history of atopy is common in dominant ichthyosis vulgaris, it seems important to us that both our patients were ichthyotic and widespread *Herpes simplex* occurred in the absence of active atopic eczema. Dominant ichthyosis vulgaris should be added to the list of skin diseases in which KVE may occur.

We should like to thank Dr P. F. Borrie for permitting us to publish details of the first patient.

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Review

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A REVIEW OF THE RECENT LITERATURE ON DISEASES OF THE VULVA—PART I. GENERAL ASPECTS

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THE material dealt with in this review is that obtained from a comprehensive culling of the recent literature for all matter relevant to the dermatologist up to late 1971. It reflects that which happens to have been written about, which may not be the same as that which we particularly need or wish to see explored. Subjects of especial significance will therefore be dealt with somewhat more fully than others.

Sometimes it has been felt desirable to note findings not strictly confined to the vulva but still of interest to the dermatologist, to report work which is for some reason based mainly on male patients but can be applied to the female, and even occasionally to report cases in the male analogous to those mentioned in the female.

Conditions of the vulval area are often difficult to interpret for the dermatologist, gynaecologist and pathologist alike because of both the specialization of their training and the differences in the terminology they employ. There may be a case, as advocated by Weisfogel (1969), for a combined approach to any given patient in a combined clinic where these specialists can find common ground.

Books

Novak's textbook (1970) provides a fairly comprehensive section on diseases of the vulva, but has relatively little to offer the dermatologist. Spelling of familiar terms is capricious, injections of alcohol and surgical cutting of nerves are mentioned as treatment of pruritus, and references are not recent. As regards pathology, Gompel and Silverberg (1970) deal with it briefly, while Novak and Woodruff (1967) are more comprehensive but lack an up-to-date edition.

Huffman's book (1968) is concerned with gynaecological conditions in childhood and adolescence. It is primarily gynaecological in approach, but its section on tumours and congenital abnormalities is useful for reference. Jones and Heller (1966) have produced a more authoritative work on paediatric and adolescent gynaecology. The chapters on embryology and anatomy and physiology are comprehensive, and the book also deals in detail with endocrine abnormalities, infections and tumours.

Lawson and Stewart (1967) have written on obstetrics and gynaecology in the tropics and in developing countries. Relevant to the present review are the descriptive accounts given of the infectious diseases encountered there, with full details of practical investigation and treatment. The book is also enlightening on differential diagnosis in such general problems as chronic lymphoedema and elephantiasis, and on acquired gynatresia which may result from female circumcision and chemical vaginitis of bizarre origin and lead to dermatitis and vulvo-vaginal infection from urinary contact. Those practising in temperate regions may on occasion meet these problems.

For sustained help over the whole field, however, the dermatologist will find the book of Gardner and Kaufman (1969) invaluable: it deals with the vagina and vulva in respect of benign conditions only, although including intraepithelial cancer. It is well written, clearly set out, extremely well illustrated, and provided with full lists of up-to-date references at the end of each chapter.

General Reviews

Readers of German will find helpful the papers of Grimmer (1967, 1968, 1969, 1970, 1971). They consist of short reviews and case reports with clinical and histological photographs and good selected references; some will be specifically mentioned later. Huffman (1970) briefly discusses paediatric problems under the title "Kindergarten gynecology", but gives no references. Altchek (1969) gives a much more informative survey of the same subject, but again without references.

Anatomy

Marshall (1970) has written a well documented account (202 references) of the adrenergic innervation of the female reproductive tract, its anatomy, physiology and pharmacology; the vulva, however, is excluded.

Lotocki (1969, 1970), in 4 papers in Polish, but with English summaries, has made a study of the neurohistology of the vulva in post-menopausal women (without controls). He finds that free nerve endings appear to degenerate more than those covered with a connective tissue sheath, that the innervation of the vessels appears to be reduced, and that in some senile tissue there is evidence of regeneration of nerves.

CONGENITAL LESIONS

Dewhurst (1968) has reviewed and illustrated congenital malformations of the genital tract, often involving the vulva, in childhood. The review is based on material seen personally. He discusses treatment and investigation, stressing that the intestinal and urinary tracts may also be involved. Of those conditions described, labial adhesions are the most likely to confront the dermatologist—and they may in fact be acquired rather than congenital. They can be separated directly, or following the application of oestrogen creams.

Nesbitt and Abdul-Karim (1968) describe 2 patients with a persistent urogenital sinus. They presented in young adult life, since a thick membrane obliterates the introitus and interferes with coitus—although one patient was in fact pregnant. The condition arises as a result of incomplete dissolution of the urogenital membrane after its rupture in the 7th week of gestation; the urethra and vagina open into the cavity behind it.

Williamson and Dewhurst (1970) describe 2 cases, niece and aunt, of testicular feminization with clitoral enlargement.

Falk and Hyman (1971) describe a woman of 25 with congenital absence of the clitoris (although the presence of clitoral tissue on microscopic examination could not be excluded). They discuss the disputed embryology of the condition, and conclude that the defect may be a milder form of that leading to extrophy of the bladder.

COMPARATIVE STUDIES

There is a virus which causes pustular vulvo-vaginitis (as well as rhinotracheitis) in cows, and Huck *et al.* (1971) report an outbreak of penoposthitis induced in bulls by this agent.

Brodey and Roszel (1967) examined 96 cases of neoplasm in the canine uterus, vagina and vulva arising in 90 animals; 85 were from the vulva and vagina. The pattern of incidence is quite different from that in humans; vaginal fibroids, not uterine fibroids, are common in dogs, for example. A major distinction is the presence in the dog of the canine transmissible venereal tumour. Adams *et al.* (1968) have studied the properties of this tumour and obtained evidence that DNA from it inhibits growth of fibroblast cultures.

The anatomy may be to some extent different, but it is of interest in connection with work on bacterial flora of the introitus in relation to urinary infection in women (O'Grady *et al.*, 1970) that Bergin and Shipley (1968) think fertility in mares may be reduced by faecal or urinary contamination of the vagina and endometrium, and that the contamination is related to anatomical factors whereby the entrance of the vagina is not adequately guarded by a normal vulvo-vaginal constrictor mechanism.

Lemke (1967) surveys the literature on carcinoma of the vulva in cattle of various types, and reports 20 cases of vulval squamous carcinoma in Kenya. The carcinomata tend to arise in non-pigmented areas of the vulva, and some types of cattle are more susceptible than others, perhaps in relation to this factor. It has been speculated that light may be important, even at this site, in inducing carcinoma, as it is in inducing carcinoma of the white skin in humans in Kenya.

CYTOLOGY

Dennerstein (1968) has studied the cytology both of the normal vulva and of the vulva in various pathological states. He describes the technique of obtaining and staining smears from various areas. His findings were checked histologically. The cells, mostly epidermal, vary somewhat from one part of the vulva to another and show cyclical changes, especially at the vestibule. Twelve cases of carcinoma (10 squamous, one basal, one metastatic from the cervix) yielded abnormal cells. Condylomata acuminata showed cells different from the carcinomata. Lichen sclerosus revealed normal cytology.

Tozzini *et al.* (1971) have made a more detailed study of the inner aspect of the labia minora only, with reference to the cyclical changes, in 10 women (8 normal, one on oestrogen, one on clomiphene) throughout one or 2 menstrual cycles, taking 2 or 3 specimens a week, and comparing the findings with the vaginal cytology. The authors found the same pattern was followed in the vagina and vulva, and suggest the method may be useful in children, virgins, or where there is vaginal infection. It would also be simple for the patient to take specimens herself. This use of vaginal cytology would seem to be more promising than its use as a substitute for biopsy.

MISCELLANEOUS DERMATOSES

Boulton Hewitt (1971) reports benefit from an oral contraceptive in a patient with scarring oral and vulval ulceration of long standing whom he regards as probably having Behçet's disease. He was encouraged to try this treatment by a report of its success in a patient with oral, vulvar and ocular ulceration whose lesions flared with menstruation. Rationalization and justification of the therapy is provided by the theory that, in some patients, Behçet's disease results from an auto-immune vasculitis arising from the patient's own endogenous progesterone, production of which will be suppressed to some extent by the contraceptive pill.

Fusion of the labia, with obstruction to the flow of urine, was reported by Damanski *et al.* (1969) as a result of lichen sclerosus, but subsequently confirmed by McCallum (1969) as being caused by benign mucous membrane pemphigoid.

Thiers *et al.* (1968) describe a case of familial benign chronic pemphigus (FBCP) localized mainly to the vulva. There was a family history and typical histology. No pemphigus antibodies were found in the serum. On examination by electron microscopy, the basal membrane was intact, the desmosomes had ruptured and the tonofibrils had lost their striations. Of uncertain significance was the finding of chromosomal breakages in mitoses of the blood cells. The patient had *Candida* at the vulva, probably coincidentally, but the authors are reminded of the work of Burns *et al.* (1967). These latter authors, with 3 cases of FBCP, found histological changes of FBCP in 2 of them under patch tests of *Candida albicans* cultures applied to the normal skin; dead *Candida* had no such effect, nor had control patches. Izumi *et al.* (1971) found that, in a patient with FBCP, histologically proven lesions could be precipitated by hydration under occlusion, tape stripping, and neomycin (the patient being allergic to neomycin). Previous workers have been able to produce lesions by trauma, and it seems that trauma, bacteria and contact sensitivity can be clearly implicated in outbreaks of FBCP in susceptible patients.

Lichen sclerosus et atrophicus (LSA) is discussed by Klostermann and Ipsen (1967) in 79 cases taken from the literature; a very large number of references are given.

Wallace (1971) gives an authoritative account of all aspects of LSA. He deals with the histology, incidence and clinical course in both sexes with particular reference to vulval lesions in women and girls, based on his own clinical material and that of others. In particular detail he discusses its relation to vulval atrophy and to leukoplakia, and the problems of terminology. His main conclusions in respect of the vulva in adults are that the areas mainly affected are the labia minora, clitoris, vestibule and introitus and the inner aspects of the labia majora; that it is the commonest cause of primary atrophy of the vulva; that leukoplakia may occur in half or more of the cases; and that carcinoma developed in 12 of 290 patients, even under conditions of careful follow-up, excision of doubtful areas, and treatment. The neoplasia in all cases developed on a leukoplakic area. In 85 patients with vulval carcinoma, 52 had LSA of the vulva. In the light of his experience he advocates local steroid treatment, or limited excision of suspicious areas, rather than indiscriminate vulvectomy. In girls before puberty, vulval lesions occurred in 42 of 50. From the patients in this series who were followed up to and after the menarche, and from the evidence of other surveys in the literature, he concludes that in about two-thirds of the cases resolution occurs by the time of the menarche. Subjective and objective improvement did not always go together, and neither could be correlated with the age of onset, initial severity or treatment.

Investigation into this disease so far has not been very rewarding. Wallace mentions electron microscope findings in extra-genital areas which appear to be distinctive, and the previous investigations by Kaufman *et al.* (1967) and by other workers with radioisotopes, which gave equivocal results. Grönroos *et al.* (1969) have carried out similar work in patients with various vulval dysplasias and malignancies and again found no definite distinction between uptake in normal and abnormal tissue; such changes as have been found may be related simply to thickening or anatomical site.

Grimmer (1968d, 1967e) notes 2 unfamiliar conditions. One he entitles apocrine miliaria of the vulva. These lesions appeared transiently in an elderly woman. He believes that clinically and histologically they are distinct from Fox–Fordyce disease. The other is vulvitis chronica plasma cellularis, a title given by Zoon, who described his balanitis plasmacellularis in 1950 and later reported 2 cases at the vulva with the same histology (Zoon, 1955). Grimmer ascribes to it a distinctive clinical appearance—glistening, wrinkled, with an arcuate edge, haemorrhagic areas and reddish-brown speckling—but a somewhat variable histology, sometimes, but not always, characterized by a dense infiltration of plasma cells He thinks it may occur alone or superimposed upon LSA. Pullè and Rigano (1968) have studied the histo-chemistry of normal and abnormal vulvar epithelium.

McCallum and Kinmont (1968) describe fully the dermatological manifestations of Crohn's disease; in 138 cases, 59 had skin lesions; 28 of these had ulceration on the anterior abdominal wall involving the genitalia. Thomas (1968) showed a case entitled " Anogenital Sinuses and Polypi, ?Crohn's Disease "; however, it appears (Thomas, 1971) that the lesions resolved and a bowel biopsy showed no evidence of Crohn's disease, so this case cannot be considered as a further definite example of the condition.

Rainford (1970) described a patient with oedema of the vulva thought to be related to pre-eclampsia; Caesarean section had to be carried out because of the vulval oedema. In the next pregnancy the oedema recurred, and Southey's tubes were inserted, with successful drainage of the fluid and normal vaginal delivery.

TREATMENT IN BENIGN VULVAL DYSTROPHIES

Two recent papers conclude that topical testosterone may be of value in benign vulval dystrophies.

Friedrich (1971) discusses earlier work on these lines, which concluded that the treatment was of value; some patients had pre- and post-treatment biopsies demonstrating improvement, and no serious side effects after follow-up in some cases of 15 years. The original contribution on this subject was by Richardson and Williams (1958), following a chance observation of improvement in the skin when testosterone was used on a phimotic clitoris. The trials, however, were not carried out in a double blind way, and Friedrich here remedies this omission, using 30 patients and comparing the effects of 2% testosterone in petrolatum with petrolatum alone. Nineteen patients, 11 with placebo, 8 with testosterone, completed a 3 months' course and were assessed in terms of histology (pre- and post-treatment biopsies very carefully matched) and relief of symptoms. No condition thought to be malignant or pre-malignant was included. The 11 who failed to complete the course consisted of one found to have cancer *in situ*, and withdrawn; the other 10 were lost to follow-up, 9 on testosterone and one on placebo; of the 9, 4 had improved in the time observed. Three patients were switched to testosterone from the placebo after 3 months, and all were helped. The amount of testosterone applied was 8–10 mg 3 times a day for the 3 months' period. Improvement could be maintained on a single application daily. No abnormalities developed on examination of breasts, pelvis, cervical smear, blood count and urine. Of the 8 on testosterone, 5 had good symptomatic relief and gross histological improvement, both atrophic and hypertrophic conditions being improved. Of the 11 on the placebo, only one had good symptomatic relief, and none histological improvement. Although 5 in this group had fair symptomatic relief, this again was not accompanied by histological changes. Five of the 8 on testosterone had increased libido and one of these had some clitoral hypertrophy and increased hair on the lip.

The author postulates that the skin in vulval dystrophy is like the thin vulval skin found in testicular feminization, where the ability to metabolize testosterone is decreased compared with the normal vulva, although the gonad itself produces testosterone; normal vulval skin *in vitro* forms dihydrotestosterone from its precursor testosterone more quickly than skin from other areas. Testosterone metabolism in the skin with reference to these points is discussed further by Hsia (1971).

Zelle (1971) has also found local testosterone propionate 2% in petrolatum helpful, but not using a double blind technique. He used it on 9 cases diagnosed as LSA, atrophic vulvitis and kraurosis, and 6 as leukoplakia without nuclear atypia and neurodermatitis. They were treated with applications 3 times a day for 6 weeks, or until better, the amount then being reduced, and were followed up for 6 months to 3 years. Frequent biopsies were taken. He finds maintenance treatment is needed. In the first, atrophic, group he found marked clinical and histological improvement; in the second, improvement was mainly subjective. Again, side effects consisted of clitoral hypertrophy and increased libido in a few patients.

For LSA, Domonkos (1969) advocates, amongst other measures, triamcinolone given by jet gun.

MISCELLANEOUS GYNAECOLOGICAL MATTERS OF DERMATOLOGICAL INTEREST

Friedman *et al.* (1971) found bullae on the cervix uteri in a patient with pemphigus vulgaris whose lesions elsewhere were suppressed by prednisone, and histology showed intraepithelial bullae and acantholytic cells. They cite a previous record by Sagher in 1970 to the effect that, in a high proportion of women with pemphigus, Nikolsky's sign can be elicited when the cervix is rubbed with gauze.

Artefactual lesions of the vagina leading to bleeding are described by Goldfield and Glick (1970) and traumatic lesions of the vagina and occasionally of the vulva by Metsälä and Nieminen (1968).

Beighton (1969) discusses obstetric aspects of the Ehlers–Danlos syndrome—bleeding after episiotomy, tearing, slow wound healing; no striae gravidarum develop. Premature delivery and a tendency to abortion is recognized in these patients.

Scully (1970) found that in a collection of 10 cases of a rare ovarian tumour, which he calls a sex cord tumour with annular tubules, 3 patients had the Peutz–Jeghers syndrome. On searching through the records of patients with the Peutz–Jeghers syndrome and ovarian tumours, they found, on examining the histology of 12 of the total of 13 tumours, that 3 were of this rare type.

Christian (1971) has further discussed the relationship between the syndrome and tumours of the ovary, attempting to define a common cause for the ovarian and intestinal lesions.

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Br. J. Derm. (1972) **86**, 648.

Books

Progress in Allergy. Volume 15. Ed. P. Kallos, B. H. Waksman and A. de Weck (1971). Basel: Karger. Pp. 486. Price £12.10.

The rate of growth of immunology is such that it is beyond the capacity of even the most avid reader to keep with the developments in the many areas into which immunology now extends. Most immunologists therefore eagerly look forward to the arrival of two annuals—*Progress in Allergy* and *Advances in Immunology*. The sheer weight of new ideas and facts in allergy is reflected in the size of this latest volume of *Progress in Allergy* which—compared with previous ones—is a bumper number.

Five of the 6 reviews cover current developments in basic immunological problems whilst the remaining one, of value to the clinician, is a review of chronic hepatitis. Vischer describes the immunological aspects of chronic hepatitis dealing in particular with the evidence that immunopathological processes are involved in certain types of hepatitis. A discussion of the possible role of viruses in experimental hepatitis is followed by accounts of the occurrence of Australia and Del Prete antigen in man.

At a more fundamental level, Elkins in a long review deals with the difficult problem of the graft *versus* host reaction and its disease which includes cutaneous manifestations. Although donor cells appear to be activated initially by the host antigen, much of the subsequent cell and tissue damage appears to be the result of immunologically non-specific cytotoxic reactions.

Voisin covers the related transplantation topic of immunological facilitation or enhancement in which, paradoxically, prior immunization with an antigen may prevent rejection of tissue bearing that antigen. This may be due to the covering of antigenic sites on the foreign antigen by enhancing antibody so that they are not recognized. The presence of antigen–antibody complexes may also suppress antibody formation.

Second exposure to an antigen results in antibody production which differs in several respects from that which occurs on first exposure. Celada concisely reviews the immunological memory processes that may be responsible for the differences. Antigen recognition by lymphocytes is dealt with in a separate review.

The production of cross-reacting antibodies may, on occasion, lead to human disease as, for example, occurs in rheumatic fever where an antibody against streptococcal antigen cross-reacts with human tissue. Springer gives a general account of the blood group and Forssman antigenic determinants shared by microbes and mammalian cells and touches on the possible hazards to man.

Some of these reviews are rather long but each is preceded by a detailed list of contents and so information is readily accessible.

J. J. Cream

Resistance to Infectious Disease. Ed. Robert H. Dunlop and Harley W. Moon (1970). Saskatoon: University of Saskatchewan. Pp. 311. Price £4.25.

The volume is the proceedings of an international symposium organized by the Western College of Veterinary Medicine of the University of Saskatchewan in Saskatoon in July 1969. The 27 invited speakers from North America and Europe range widely, some giving review articles, others concentrating on their personal contributions to the subject. They are, of course, concerned with many different animal hosts and numerous infective agents.

In the first chapter F. B. Hutt discusses genetic factors in resistance to infection. Here there is one item to interest dermatologists especially, in that he draws attention to the work of Adams and Rickard on the anti-streptococcal activity of bovine teat canal keratin. Resistance to streptococcal infection apparently depends on the fatty acid content of this material and this is determined by genetic factors. Hilton B. Levy reviews his own work on interferon and its mode of action, and discusses non-replicating interferon inducers. Lars Karstad in a useful paper, discusses transplacentally transmitted virus diseases and reminds us that they may mimic genetic disorders. Newberne and Williams' paper on nutritional influences in infections and A. J. F. Webster's paper on the physical factors influencing susceptibility to infection underline the difficulties inherent in both these fields.

One of the most stimulating chapters is that on microbial interactions on body surfaces and its relevance to resistance to infectious disease from Dwayne Savage and Julia Sue Mac-Allister of Texas. The authors are concerned primarily with the gut, but their ideas may well have relevance on skin. They discuss the phenomenon of layering of organisms on the surface of mucous membranes, a topic of importance to stomatologists if not to dermatologists generally.

G. D. Abram's contribution on the effect of the microbial flora on mechanisms of host resistance is an excellent introduction to work which has been done in germ-free animals. He concerns himself mainly with the effect of gut organisms on the epithelium of the ileum, but the parallels which might be drawn in other sites are obvious. H. Platt covers the skin specifically in his account of factors contributing to resistance and susceptibility in stratified squamous and respiratory epithelia.

H. Smith of Birmingham discusses host factors, other than immunological ones, which may influence microbial proliferation *in vivo*. His paper is concerned with such factors as the pH of body tissues, temperature, concentrations of nutrients and the effects of these growth conditions on the production of agressins by bacteria.

E. F. Anderson's chapter will be most welcome to those readers who want a good account of transferable resistance, and might well be marked as essential reading for all who prescribe antibiotics.

There are several papers on immunology in the accepted sense, including a critical one by Cottier and his associates from Berne. They discuss proliferation and differentiation of macrophages and lymphocytes. J. G. Hall in a paper illustrated by some striking photomicrographs also discusses lymphocytes. There are also contributions from Willoughby on mediators of vascular responses, and from R. J. Downey on granulocyte function, the latter providing a very readable account of this subject. Much that is in this volume will be familiar to those interested in infectious disease; most of it has little immediate relevance to dermatology. However, it does provide a readable and in places stimulating review of the subject. As a valuable corrective to narrowness of vision and myopia it can be recommended to all concerned with cutaneous microbiology or the care of patients with skin infections.

STEPHEN ROBERTS

Br. J. Derm. (1972) **86**, 650.

Correspondence

An Assessment of Dapsone Toxicity in the Guinea-pig

SIR,—Prompted by the neuropathy apparently due to dapsone in the patient described by Wyatt and Stevens (1972) we made a small assessment of the effect of dapsone on the peripheral nerves of guinea-pigs. Six female adult albino guinea-pigs (body weight approximately 500 g) received twice weekly intramuscular injections of 4,4-diaminodiphenylsulfone (dapsone) in arachis oil for 12 weeks. Four received a dose of 30 mg/kg body weight/week, and 2 received a lower dose of 3 mg/kg body weight/week. The dose in the above patient was approximately 20 mg/kg body weight/week. Control guinea-pigs received injections of arachis oil alone. All animals were sacrificed after 12 weeks. Histological sections of the lumbar spinal cord, dorsal root ganglia, dorsal and ventral roots, sciatic, posterior tibial and digital nerves, and histological and histochemical preparations of the hamstring, gastrocnemius and plantar muscles were examined.

Neither clinical signs nor histological changes of a neuropathy were observed in any of these animals.

The dosage used in these animals was somewhat above that required to control the rash in most patients with dermatitis herpetiformis, and well above that used in leprosy. Potential differences in sensitivity between different species make interpretation difficult, but the absence of nerve damage in the guinea-pigs would be compatible with the conclusion that the neuropathy in man is ideosyncratic rather than dose-dependent.

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REFERENCE

WYATT, E. H. & STEVENS, J. C. (1972) Dapsone Induced Peripheral Neuropathy. *Br. J. Derm.*, **86**, 521.

Correction

In the letter by Drs Portnoy and Molokhia (*Br. J. Derm.*, 1972, **86**, 205) there is an error in Tables I and II. The severity of psoriasis grading should have been recorded as:

Severe (more than 10%)
Minor (less than 5%)

Br. J. Derm. (1972) **86**, 651

Quarterly Review

DRUG REACTIONS XVIII

Cutaneous Reactions to Antituberculous Drugs

Antituberculous drugs are usually prescribed in combinations of at least 2, and often 3, so that the emergence of bacterial resistance may be prevented or delayed. As a result of this, it is often difficult to distinguish which drug causes particular side effects. With past experience, and usually only after the suspected drug has been withdrawn and given in test doses, is it possible to find which is responsible. Often, side effects develop during the first 6 to 12 weeks of treatment, but may occur at any time.

Treatment of tuberculosis can be divided into that with standard drugs, and that with reserve or second-line drugs. Standard treatment usually involves the use of streptomycin, para-amino salicylic acid and isoniazid. The reserve drugs now used in clinical practice include thiacetazone, ethionamide, prothionamide, pyrazinamide, cycloserine, kanamycin, viomycin, capreomycin, and the rifamycins. Thiacetazone is now used by some as a standard drug. These second-line drugs are rarely used in initial treatment because they are in general more toxic and less effective than the first-line drugs, but are kept in reserve where initial treatment with standard therapy has failed.

Standard Chemotherapy

The reported frequency of reactions varies considerably, but hypersensitivity to streptomycin and P.A.S. appears to be more common than to the other drugs. A high incidence of eczematous eruptions has been reported in patients receiving various drug combinations (Becker and Mounce, 1957). Crissey *et al.* (1957) described a group of patients who developed eczematous eruptions under treatment with dihydrostreptomycin, aminosalicylic acid and isoniazid in various combinations. Selective withdrawal of the drugs incriminated aminosalicylic acid as the chief cause, dihydrostreptomycin as an occasional cause and isoniazid as not being involved. Agrawal (1969) reported a patient who showed extreme hypersensitivity to almost all antituberculous drugs except ethambutol. Attempts to desensitize him were unsuccessful.

Streptomycin.—Exanthematic eruptions develop in 5 to 10% of patients, usually associated with fever. Allergic reactions are usually of the delayed type, and are characterized by intense pruritus. Epidermal tests with 0·1% streptomycin take a correspondingly long time to become positive and should be read after 3 to 4 days (Bolgert, 1965). Immediate reactions, such as anaphylactic shock, occur but are rare. If the antibiotic is continued there is a risk that exfoliative dermatitis may develop (Harris and Walley, 1950; Peterkin, 1956). Skin sensitization is also common in nurses and dispensers who handle streptomycin and this may lead to severe eczema, sometimes associated with periorbital swelling and conjunctivitis. Desensitization of those who handle streptomycin is usually possible but may take several months and quite severe reactions may occur even with the minute doses used. Patients showing hypersensitivity during therapy are generally more readily desensitized often under cover of a corticosteroid.

Stomatitis, which may be severe (Belham and Perr, 1948), is occasionally reported and ulcers in the mouth may be the presenting symptom of agranulocytosis. Other manifestations which have been reported are purpura and bullous lesions (Peterkin, 1956) and lichenoid eruptions (Renkin, 1958). Induration may occur at the site of injections of streptomycin, usually due to the drug having been given subcutaneously. Occasionally, it is due to the patient being sensitive to the sulphate and this can then be avoided by using the calcium chloride salt (*Br. med. J.*, 1963).

Para-aminosalicylic acid (*P.A.S.*).—Hypersensitivity reactions to P.A.S. are common. Fever, rash, gastrointestinal complaints and occasionally lymphadenopathy comprise the usual picture. Macular and urticarial rashes have been reported (Ahmed, 1963). P.A.S. has also been mentioned as producing an infectious mononucleosis-like syndrome, comprising general malaise, fever, headache, rash, lymphadenopathy, splenomegaly and an increase in the count of atypical lymphocytes. However the Paul Bunnell test is negative (Sternberg and Bierman, 1963). Anaphylactic reactions are rare but have been reported with antibodies to P.A.S. demonstrated in some cases (Véran *et al.*, 1962). P.A.S. may cause reactions similar to those due to sulphonamides, since it has a para-amino group, but sometimes the salicylic acid part of the molecule is responsible. Peterkin (1956) described a child who developed non-thrombocytopenic purpura which he attributed to the salicylic acid part of the molecule. The rash was reactivated by aspirin.

P.A.S. can also cause an exfoliative dermatitis (Griffiths and Watts, 1965) and this occurs more commonly than with the other antituberculous drugs. Other reported cutaneous manifestations attributed to P.A.S. include a fixed drug eruption involving the penis (Kierland and Carr, 1949), lichenoid eruptions (Shatin *et al.*, 1953) and a lupus erythematosus-like syndrome (Sternberg and Bierman, 1963).

Isoniazid.—Isoniazid has a low incidence of side effects if used in a low dosage of up to 5 mg per kg body weight. Allergic reactions such as urticaria, and angioneurotic oedema occur in a small proportion of patients. Eruptions due to isoniazid are often morbilliform and rarely progress to exfoliative dermatitis (Honeycutt and Huldin, 1963). Pruritus without skin eruption is not unusual, and flushing of the face has been reported several times (Witbind and Willner, 1953). An acneiform eruption is seen frequently in patients receiving isoniazid, particularly in adolescents and young adults with a seborrhoeic tendency (Hesse, 1966).

The appearance of pellagra-like signs in patients is well documented (Aspinall, 1964; Honeycutt and Huldin, 1963). It has been suggested that isoniazid blocks the action of nicotinamide by competitive inhibition. Isoniazid resembles nicotinamide structurally, differing from it in the side chain of the pyridine nucleus (Sachdera *et al.*, 1965). Biehl and Vilter (1954) showed that disorganization of pyridoxine metabolism occurs during isoniazid medication. However, Harrison and Feiwel (1956) described a patient who developed pellagra while receiving isoniazid even though pyridoxine was also given. Occasionally a lupus-erythematosus syndrome has been reported. A patient with rheumatoid arthritis was given isoniazid and developed a lupus-like syndrome with pericardial effusions; antinuclear factor and lupus erythematosus cells were present. These symptoms and signs cleared after isoniazid was discontinued (Hothersall *et al.*, 1968). Other similar cases have been recorded (Jadassohn *et al.*, 1965; Sternberg and Bierman, 1963). Cannat and Seligmann (1966) found an abnormally high incidence of antinuclear factor in tuberculous patients, being treated with tuberculostatic agents. They found no relationship to duration of the disease and noted that isoniazid was the only drug common to all patients.

Other reported side effects include non-thrombocytopenic purpura (Collins, 1952; Duncan, 1964) and a patient with striae cutis atrophica (Hoefer, 1967).

Second Line Drugs

Thiacetazone.—Side effects from this drug include pruritus, flushing and non-specific morbilliform rashes. Two cases of Stevens–Johnson syndrome have been reported (Seth and Mukherjee, 1966).

Ethionamide.—Rather similar side effects to those seen with isoniazid are experienced. In a comparison of two groups treated with these drugs, the ethionamide group showed more serious toxicity (Schwartz, 1966). Allergic reactions to ethionamide are reported occasionally. Urticaria was reported by Schütz (1961). Vomiting, followed by pyrexia and a generalized punctate erythematous rash was recorded in a woman, who was subsequently shown to be allergic to ethionamide when she received the drug again (Carey, 1965).

A confluent seborrhoeic rash (Brouet *et al.*, 1961) mainly localized to the forehead, is one of the most frequent skin manifestations. Acneiform eruptions have been reported on several occasions (Brouet *et al.*, 1961; De Voogd and Mourney, 1961). A tendency to photosensitivity with erythema and pigmentation on exposed skin has been described (De Voogd and Mourney, 1961; Friedmann, 1966). Simon (1962) reports a patient who developed a morbilliform erythematous eruption initially confined to exposed parts but later becoming generalized. Severe ichthyosis occurred during the treatment of a patient with tuberculous meningitis and regressed when ethionamide was withdrawn (Moulin *et al.*, 1964). Occasionally stomatitis develops and alopecia has been reported (Baran, 1966).

Prothionamide.—Prothionamide is the n-propyl derivative of ethionamide. Most of the adverse reactions mentioned under ethionamide are seen during therapy with prothionamide. Of these an acneiform eruption has been reported on several occasions (Bariéty *et al.*, 1966).

Reports of cutaneous side effects to the remainder of the second-line antituberculous drugs are less common. *Pyrazinamide* may produce exanthematous rashes (Petty and Mitchell, 1962) and less frequently photosensitivity with bright reddish-brown discolouration of exposed skin (Somner and Brace, 1962; Pines, 1965). *Cycloserine* was reported to produce a purplish scaly eruption (Warnery *et al.*, 1957). Sensitization to *Kanamycin* seems to be less frequent than to streptomycin (Kreis, 1966). Hypersensitivity reactions may occur due to the previous use of neomycin or framycetin in dermatological preparations, *i.e.* cross allergy. *Viomycin* is more toxic than streptomycin, renal damage being the most serious side effect. Generalized urticaria and eczema have been reported (Bloedner, 1967). *Capreomycin* closely resembles streptomycin in the type, frequency, and intensity of side effects. Maculopapular rashes occur in about 6% of patients. Drug fever is mentioned with or without a rash (Donomae, 1966). Painless nodules at the site of injection are seen in many patients (Browning and Donnerberg, 1966). Toxic effects arising from *Ethambutol* are mainly confined to visual disturbances but Jesiotr (1969) reported a patient who developed purpura. No classification as to whether its origin was thrombocytopenic or vasculitic was made. The incidence of allergic reactions following the parenteral administration of *Rifamycin* has been found to be low. Berganini and Fowst (1965) reported a 0·18% occurrence. They also noted that a transient yellow discolouration of the skin and mucous membranes may occur from the intense orange-yellow colour of Rifamycin solution. *Rifampicin* (the oral form of the rifamycins) has few reported side effects and is a more effective antibiotic.

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INDEX

SUBJECTS

Acetylcholine induced response of the human sweat gland; effects of propranolol on (letter), 206
Acne; antibiotics in, Supp. 8, 5
—; bacteriology of, Supp. 8, 3
— vulgaris; effect of topical tetraynoic acid on the seborrhoea of, 66
— —; is sulphur helpful or harmful in?, 620
— —; relevance of skin surface lipids to, Supp. 8, 10
— —, sebum excretion rate and skin surface lipid composition; effect of a medicated wash on (letter), 311
Acrokeratosis verruciformis of Hopf with steatocystoma multiplex and hypertrophic lichen planus, 91
Addendum, 441
Adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin.
  I. Measurement and properties, 141
  II. Levels in atopic dermatitis, 147
β-Adrenergic stimulation; response of the sweat glands to, 282
Allopurinol, pyrazolone and derivatives; incrimination of in toxic epidermal necrolysis, 564
Alopecia areata, associated with chronic mucocutaneous candidiasis and endocrine deficiency, 238
Amyloidosis cutis, macular (letter), 203
Antibiotics in acne, Supp. 8, 5
Anticholinergic agent and iontophoresis of tap water in the treatment of idiopathic hyperhidrosis, 72
— —; effect of prolonged topical application of on the sebaceous glands, 61
Antihistamine (Incidal) and prednisone; a study of the effect of on patch test reactions, 68
Antimetabolites and psoriasis, 87
Antimycotic agent: BAY b 5097, topical treatment with, 628
Antituberculous drugs; cutaneous reactions to, 651
Appointments, 447, 554
Atopy and ichthyosis vulgaris, 1

Bacteriology:
  bacteriology of acne, Supp. 8, 3
  tetracycline-resistance β-haemolytic streptococci in dermatological patients, 449
Barbiturates; cutaneous reactions to, 313
BAY b 5097, a new broad spectrum antimycotic agent; topical treatment with, 628
Biochemistry: activity of metabolic enzymes of human epidermis in relation to age, 134
  adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin,
  I. Measurement and properties, 141
  II. Levels in atopic dermatitis, 147
  collagen, collagenase and clinicians, 193
  control of glycogen utilization in skin; phosphorylase B kinase and phosphorylase A phosphatase, 49
  correlation between various proteins of bovine snout epidermis, 495
  fluorimetric assay of carboxylic ester hydrolases in guinea-pig skin and the effects of propranolol, 606
  lipid synthesis (^{14}-acetate incorporation) in the isolated human sebaceous gland, the appendage-freed epidermis, the sebocyst (steatocystoma) and the wen (keratinous cyst of skin), 614
  variations in skin surface lipid composition and sebum excretion rate with different sampling techniques, II, 356
Book reviews:
  A Treatise of Skin. Vol. 1—Biophysical Properties of the Skin, ed. H. R. Elden, 308
  Acne Vulgaris, S. B. Frank, 309
  Colours for Cosmetics. 3rd Report of the Farbstoff-Komission of the Deutsche Forschungsgemeinschaft, 443
  Common Skin Diseases, H. T. Behrman, T. A. Labow and J. H. Rozen, 103
  Cutane Paraneoplastische Syndrome, ed. J. J. Herzberg, 309
  Dermatology in General Medicine, ed. T. B. Fitzpatrick, K. A. Arndt, W. H. Clark, A. Z. Eisen, E. J. Van Scott and J. H. Vaughan, 549
  Epidermolysis Bullosa, T. Gedde-Dahl, 443
  Human Hair Growth in Health and Disease, D. Ferriman, 208
  Iatrogenic Diseases, P. F. D'Arcy and J. P. Griffin, 442
  Progress in Allergy, Vol. 15, ed. P. Kallos, B. H. Waksman and A. de Weck, 648
  Resistance to Infectious Disease, ed. R. H. Dunlop and H. W. Moon, 648
  Sjögren's Syndrome, M. A. Shearn, 442

Symposium on Pediatric Dermatology, ed. A. H. Jacobs, 208
The Significance of Physical Signs in Medicine, P. Mills, 444
Year Book of Dermatology, ed. F. D. Malkinson and R. W. Pearson, 549

Candida albicans, alimentary; and napkin rashes, 458
— — infection and napkin rash (letter), 207
— —, spores of, and dermatophytes; distribution of in the environment, Supp. 8, 69
Candidiasis, chronic mucocutaneous: associated abnormalities of iron metabolism, Supp. 8, 88
—, — —, with endocrine deficiency and alopecia areata, 238
Capreomycin; cutaneous reactions to, 653
Carbon dioxide release by small skin areas; a micro method for measuring, 463
Carboxylic ester hydrolases; fluorimetric assay of in guinea-pig skin and the effects of propranolol, 608
Carcinoma, eccrine sweat gland, with metastases, 95
Case Reports, 91, 175, 177, 636
Cells: epidermal cells, adult, in monolayer culture; ultrastructure of, 27
  — —, human, in DNA synthesis; a comparison of *in vivo* and *in vitro* methods of identification, 506
  keratinocytes: nuclear and nucleolar structure during the late stages of normal human keratinocyte maturation, 601
  "lymphocyte transformation" (letter), 103
Chalone control of mitotic activity in eccrine sweat glands, 586
—s; role of in epidermal homeostasis, 543
Chloral hydrate; cutaneous reactions to, 315
ω-Chloroacetophenone and *o*-chlorobenzylidenemalononitrile; cutaneous reactions produced by, when applied directly to the skin of human subjects, 150
Chlorpromazine; increased *in vitro* radiosensitivity of malignant melanoma induced by the *in vivo* administration of, 491
Cocci, aerobic, in acne, Supp. 8, 4
Collagen, collagenase and clinicians, 193
Comment, 87, 193, 302, 417, 543
Contraceptive, oral, and variegate porphyria, 453
Copper and zinc in psoriasis (letter), 205, 439
Correction, 447, 650
Correspondence, 102, 203, 310, 437, 650
Corynebacterium acnes, Supp. 8, 4
Cosmetic camouflage in dermatology, 530
Cycloserine; cutaneous reactions to, 653
Cyst of skin, keratinous—see Wen

Dapsone (letter), 312
— in the treatment of herpetiform pemphigus foliaceus (case), 99
—, inducing peripheral neuropathy, 521
— toxicity in the guinea-pig; an assessment of (letter), 650
Depigmenting action of 8-hydroxyquinoline on hair growth in the mouse, 472
Dermatitis, atopic; conditioning of scratch responses in, 574
—, —: levels of adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin in, 147
—, —; suppressed cell-mediated immunity in two adults with, 317
—, contact: allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp.), 568
  ancillary aids in elucidation of causes of contact dermatitis, 445
  cement dermatitis and atmospheric parameters in Kuwait, 155
  chromium, 106
  flavours, 105
  medicaments, 104
  paraphenylenediamine, 105
  photocontact dermatitis due to Disperse Blue 35 dye, 264
  reactions to contact allergens given orally or systemically, 104
  thiuram chemicals, 105
— herpetiformis (Duhring's disease); ultrastructural aspects of, 348
— —; improvement with gluten-free diet (case), 200
— —, juvenile, in Jamaica: clinical and gastrointestinal features, 226
— —; prevalence of (letter), 437
—, photocontact: investigation of in a dye manufacturing process, 264
—, rosacea-like: contraindication or intolerance reaction to strong steroids, 253
Dermatology; hyperbaric oxygen therapy in, 631
— in Bristol and the West of England; a history of, 180
Dermatomyositis; mycosis fungoides with (case title), 101
Dermatophytes and Candida spores in the environment; distribution of, Supp. 8, 69
Dichloralphenazone; cutaneous reactions to, 315
Diphtheroids, cutaneous; microbiology of, Supp. 8, 16
Dithranol; action of zinc oxide on (letter), 203
DNA synthesis; comparison of *in vivo* and *in vitro* methods of identifying human epidermal cells in, 506
Dressings, occlusive and semipermeable; effect

of on the mitotic activity of normal and wounded human epidermis, 593
Drug eruption, fixed (bullous), due to phenolphthalein; demonstration of intercellular immunofluorescence and epidermal hysteresis in, 118
— —, —; due to phenazone in a hypnotic (letter), 438
Drug Reactions XVII, 313; XVIII, 651
Duhring's disease—see Dermatitis herpetiformis
Dye, Disperse Blue 35; photocontact dermatitis due to, 264

Eczema and psoriasis; " Metosyn " in the treatment of (letter), 310
— herpeticum, recurrent; associated with ichthyosis vulgaris, 638
Elastosis perforans serpiginosa, disseminated, in four mongoloids, 9
Electron microscopy: a new ultrastructural finding in oral submucous fibrosis, 286
  electron microscopy of collagen, 195
  gastro-intestinal features in dermatitis herpetiformis in Jamaica, 226
  intracellular localization of tyrosinase inhibitor in amelanotic and melanotic malignant melanoma, 385
  nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte maturation, 601
  ultrastructural aspects of dermatitis herpetiformis (Duhring's disease), 348
  — differences in human malignant melanomata, 395
  ultrastructure of adult epidermal cells in monolayer culture, 27
— — (scanning): progressive changes in cuticular pattern along the shafts of human hair as seen by scanning electron microscopy, 379
  scanning electron microscopy of the stratum corneum, 272
Emotions and the skin: the conditioning of scratch responses in cases of atopic dermatitis, 574
Endocrine deficiency, associated with chronic mucocutaneous candidiasis and alopecia areata, 238
Enzymes: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin.
  I. Measurement and properties, 141
  II. Levels in atopic dermatitis, 147
  metabolic enzymes of human epidermis; activity of in relation to age, 134
Epidermal damage by surface active agents; physico-chemical factors influencing, 361
— " turnover time "—a re-examination, 14
Epidermis, appendage-freed; lipid synthesis (^{14}-acetate incorporation) in, cf. that in isolated human sebaceous gland, sebocyst and wen, 614
—, bovine snout; correlation between various proteins of, 495
—, human; activity of metabolic enzymes of in relation to age, 134
—, —, normal and wounded: effect of occlusive and semipermeable dressings on the mitotic activity of, 593
Epitheliomata, basal cell, extensive: combined cytostatic and surgical treatment of, 408
Ethambutol; cutaneous reactions to, 653
— in the treatment of tuberculosis, 211
Ethchlorvynol; cutaneous reactions to, 315
Ethionamide; cutaneous reactions to, 652

FAPG, fluocinonide in; in the treatment of psoriasis and eczema (letter), 310
Fibrosis, oral submucous; a new ultrastructural finding in, 286
Fluocinonide (" Metosyn ") in the treatment of psoriasis and eczema (letter), 310
Fluorimetric assay of carboxylic ester hydrolases in guinea-pig skin and the effects of propranolol, 608
Foetal tissues, human: quantitation of keratinization in, 508
Franco-British meeting, Toulouse 1970, 306
Fungal infections: alimentary *Candida albicans* and napkin rashes, 458
  Candida albicans infection and napkin rash (letter), 207
  candidiasis, chronic mucocutaneous: associated abnormalities of iron metabolism, Supp. 8, 88
  —, — —, with endocrine deficiency and alopecia areata, 238
  distribution of dermatophytes and Candida spores in the environment, Supp. 8, 69
  epidemiology of animal ringworm in man, Supp. 8, 78
  Microsporum equinum infection of the scalp in an adult, 175
  topical treatment of fungal infections with BAY b 5097, 628

Glands of Montgomery, 126
Gluten-free diet in the treatment of dermatitis herpetiformis (case), 200
Glycogen utilization in skin, control of; phosphorylase B kinase and phosphorylase A phosphatase, 49
Granuloma annulare in identical twins, 636
Grenz ray treatment of recurrent herpes simplex, 172

Hair growth in the mouse; selective depigmenting action of 8-hydroxyquinoline on, 472

—, human: progressive changes in cuticular pattern along the shafts of, as seen by scanning electron microscopy, 379
—s: white scalp hairs turning black—an unusual reversal of the ageing process, 513
Hendersonula toruloidea infection (case), 98
Herpes simplex, recurrent; Grenz ray treatment of, 172
— — virus infection, experimental zosteriform; in mouse skin, 40
Histochemistry: histochemical applications of skin surface biopsy, 20
  intracellular localization of tyrosinase inhibitor in amelanotic and melanotic malignant melanoma, 385
History of Dermatology, 533
Homeostasis, epidermal; role of chalones in, 543
8-Hydroxyquinoline; selective depigmenting action of on hair growth in the mouse, 472
Hydroxyurea and psoriasis (letter), 102
Hyperbaric oxygen therapy in dermatology, 631
Hyperhidrosis, idiopathic; treatment of with iontophoresis of tap water and poldine methosulphate, 72
—, localized unilateral—a clinical and laboratory study, 374
—; treatment of with topical propantheline (letter), 441
Hypoparathyroidism; associated with oral and cutaneous candidiasis, 238

Ichthyosis vulgaris, atopy and, 1
— —, autosomal dominant, 1
— — in Israel; a survey of, 1
— —, out-patient treatment of: a double-blind trial of ointments, 291
— —; recurrent eczema herpeticum associated with, 638
— —, X-linked, 1
Immunity, suppressed cell-mediated, in two adults with atopic dermatitis, 317
Immunology: demonstration of basal cell immunofluorescence in drug-induced toxic epidermal necrolysis, 246
  demonstration of intercellular immunofluorescence and epidermal hysteresis in bullous fixed drug eruption due to phenolphthalein, 118
  extraction of pemphigus antibodies from a lymphoid neoplasm and its possible relationship to pemphigus vulgaris, 411
  factors contributing to occasional failures in the laboratory diagnosis of bullous pemphigoid by indirect immunofluorescence, 111
  immunology of streptococcal infection, Supp. 8, 62
  suppressed cell-mediated immunity in two adults with atopic dermatitis, 317
Impetigo contagiosa in the United Kingdom, Supp. 8, 48
Infection, streptococcal; immunology of, Supp. 8, 62
Iontophoresis of tap water and poldine methosulphate in the treatment of idiopathic hyperhidrosis, 72
Irish Dermatological Society, 200
Iron metabolism, abnormalities of; associated with chronic mucocutaneous candidiasis, Supp. 8, 88
Isoniazid; cutaneous reactions to, 652

Jadassohn tumour arising at smallpox vaccination site, 177

Kanamycin; cutaneous reactions to, 653
Keloids, coiffure, 415
Keratinization; quantitation of in human foetal tissues, 508
Keratinocyte, normal human: nuclear and nucleolar ultrastructure during the late stages of maturation, 601

Lichen planus, hypertrophic, and steatocystoma multiplex; acrokeratosis verruciformis of Hopf with, 91
— —; the pathogenesis of, 302
Lipid, skin surface, sebum excretion rate and acne; effect of a medicated wash on (letter), 311
—, — —; variations in composition and sebum excretion rate with different sampling techniques, II, 356
—, — —; relevance of to acne vulgaris, Supp. 8, 10
— synthesis (^{14}-acetate incorporation) in the isolated human sebaceous gland, the appendage-freed epidermis, the sebocyst (steatocystoma) and the wen (keratinous cyst of skin), 614
" Lymphocyte transformation " (letter), 103
Lymphoma: a variant of pityriasis lichenoides with histological features simulating a, 329

Mebhydrolin napadisylate and prednisone; a study of the effect of on patch test reactions, 68
Medicated wash; effect of on acne, sebum excretion rate and skin surface lipid composition (letter), 311
Melanoma, amelanotic and melanotic malignant: intracellular localization of tyrosinase inhibitor in, 385
—, malignant; increased *in vitro* radiosensitivity of, induced by the *in vivo* administration of chlorpromazine, 491

Melanomata, human malignant: ultrastructural differences in, 395
—, malignant, in lymphoedematous arm following radical mastectomy for breast carcinoma (an extension of the syndrome of Stewart and Treves) (case title), 101
Microbiology of the cutaneous diphtheroids, Supp. 8, 16
Microsporum equinum infection of the scalp in an adult, 175
Mitotic activity in eccrine sweat glands; chalone control of, 586
— — of normal and wounded human epidermis; effect of occlusive and semipermeable dressings on, 593
Mongolian spots in negro children from the West Indies, 260
Mottling of the skin, congenital diffuse, 515
Mycosis fungoides, human: generalized cutaneous eruption in a dog, with histological similarity to, 164
— — with dermatomyositis (case title), 101

Nail dystrophy, psoriatic; treatment of, 79
—, finger; pathology of in psoriasis, 555
Napkin rash; *Candida albicans* infection and (letter), 207
— — and alimentary *Candida albicans*, 458
Naevus comedonicus; retinoic acid in the treatment of, 528
Neoplasm, lymphoid: extraction of pemphigus antibodies from, and its possible relationship to pemphigus vulgaris, 411
Nephritis, acute, and streptococcal pyoderma in Trinidad, Supp. 8, 55
Nerves: innervation of sweat glands in horses and dogs, 160
Neuropathy, peripheral; dapsone induced, 521
News and Notices, 108, 316, 447, 554, 656

Obituary: Brain, Reginald T., 435
Coburn, James G., 192
Smith, Archibald G., 436
Ointments; double-blind trial of in the treatment of ichthyosis, 291
Organ culture: action of staphylococcal epidemolytic toxin on mouse skin in organ culture, Supp. 8, 40
the growth of adult human skin *in vitro*, 481
Oxygen therapy, hyperbaric, in dermatology, 631

Para-aminosalicylic acid; cutaneous reactions to, 652
Patch test reactions; a study of the effect of prednisone and antihistamine on, 68
Pemphigoid, bullous; factors contributing to occasional failures in the laboratory diagnosis of by indirect immunofluorescence, 111
Pemphigus antibodies; extraction of from a lymphoid neoplasm and its possible relationship to pemphigus vulgaris, 411
— foliaceus, herpetiform; responsive to dapsone (case), 99
— vulgaris: extraction of pemphigus antibodies from a lymphoid neoplasm and its possible relationship to pemphigus vulgaris, 411
Phenazone, causing a fixed drug eruption (letter), 438
Phenolphthalein; demonstration of intercellular immunofluorescence and epidermal hysteresis in bullous fixed drug eruption due to, 118
Phosphorylase B kinase and phosphorylase A phosphatase; the control of glycogen utilization in skin, 49
Pityriasis lichenoides chronica, 215
— — et varioliformis acuta, 215
— —, " lymphomatoid "; a variant with histological features simulating a lymphoma, 329
Poldine methosulphate and iontophoresis of tap water in the treatment of idiopathic hyperhidrosis, 72
— methyl-methosulphate; effect of prolonged topical application of on the sebaceous glands, 61
Porphyria cutanea tarda treated with sodium bicarbonate (case), 200
—, variegate, and the oral contraceptive, 453
Porton jet injector, in the treatment of psoriatic nail dystrophy with triamcinolone acetonide solution, 79
— — —; penetration and dose of injections with, 83
Pre-cancer and the skin, 417
Prednisone and an antihistamine; a study of the effect of on patch test reactions, 68
Propantheline, topical; treatment of hyperhidrosis with (letter), 441
Propranolol; effects of on acetylcholine induced response of the human sweat gland (letter), 206
—; effects of on carboxylic ester hydrolases in guinea-pig skin, 608
Proteins, various, of bovine snout epidermis; correlation between, 495
Prothionamide; cutaneous reactions to, 653
Psoriasis and antimetabolites, 87
— and eczema; " Metosyn " in the treatment of (letter), 310
— and hydroxyurea (letter), 102
—: epidermal "turnover time" in, 14
—; pathology of the finger nail in, 555
—; retinoic acid in the treatment of, 524
—; treatment of nail dystrophy in, 79
—; turkey diet regime in the treatment of (letter), 440

—; zinc and copper in (letter), 205, 439
Pyoderma, streptococcal, and acute nephritis in Trinidad, Supp. 8, 55
Pyrazinamide; cutaneous reactions to, 653
Pyrazolone and derivatives, and allopurinol; incrimination of in toxic epidermal necrolysis, 564
Pyrethrin II; role of in allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp.), 568
Pyrethrosin; role of in allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp.), 568
Pyrethrum (*Chrysanthemum* spp); allergic contact dermatitis from, 568

Radio-sensitivity, increased *in vitro*, of malignant melanoma induced by the *in vivo* administration of chlorpromazine, 491
Retinoic acid in the treatment of naevus comedonicus, 528
— — in the treatment of psoriasis, 524
Rifampicin and rifamycin; cutaneous reactions to, 653
— in the treatment of tuberculosis, 210
Ringworm, animal; epidemiology of in man, Supp. 8, 78
Royal Society of Medicine, 98

Scleredema of Buschke (case title), 101
Scleroderma, linear melorheostotic, 297
Scratch responses in cases of atopic dermatitis; the conditioning of, 574
Sebaceous glands; effect of prolonged topical application of an anticholinergic agent on, 61
— —, human, isolated; lipid synthesis (^{14}-acetate incorporation) in, cf. that in appendage-freed epidermis, sebocyst and wen, 614
Sebocyst (steatocystoma); lipid synthesis (^{14}-acetate incorporation) in, cf. that in isolated human sebaceous gland, appendage-freed epidermis and wen, 614
Seborrhoea of acne; effect of topical tetraynoic acid on, 66
Sebum excretion; effect of topical tetraynoic acid on, 64
— — rate, acne vulgaris and skin surface lipid composition; effect of a medicated wash on (letter), 311
— — — and skin surface lipid composition; variations in with different sampling techniques, II, 356
Shakespeare on the skin, 533
Skin, adult human; growth of *in vitro*, 481
— disease, microbial, Supp. 8
—; emotions and: the conditioning of scratch responses in cases of atopic dermatitis, 574
— markings in negro children from the West Indies, 260
—; pre-cancer and the, 417
—; Shakespeare on the, 533
— surface biopsy; histochemical applications of, 20
Smallpox vaccination site; Jadassohn tumour arising at, 177
Sodium bicarbonate in the treatment of porphyria cutanea tarda (case), 200
Staphylococci, coagulase-negative; pathogenic role of, Supp. 8, 42
—; colonization with in dermatology wards with different designs and practices, Supp. 8, 21
Steatocystoma, 614
— multiplex and hypertrophic lichen planus; acrokeratosis verruciformis of Hopf with, 91
Steroids; contraindication to in rosacea-like dermatitis, 253
Stratum corneum; scanning electron microscopy of, 272
Streptococci, tetracycline-resistant β-haemolytic; in dermatological patients, 449
Streptomycin; cutaneous reactions to, 651
Sulphur; is it helpful or harmful in acne vulgaris?, 620
Surface active agents; physico-chemical factors influencing epidermal damage by, 361
Sweat gland, eccrine: carcinoma of, with metastases, 95
— —, —: chalone control of mitotic activity in, 586
— —, human; effects of propranolol on the acetylcholine induced response of (letter), 206
— —s in horses and dogs; innervation of, 160
— —; response of to β-adrenergic stimulation, 282

Tetraynoic acid, topical; effect of on seborrhoea of acne, 66
— —, —; effect of on sebum excretion, 64
Therapeutics XVII, 210
Thiacetazone; cutaneous reactions to, 652
— in the treatment of tuberculosis, 212
Toxic epidermal necrolysis, drug-induced; demonstration of basal cell immunofluorescence in, 246
— — —; further experiences of, incriminating allopurinol, pyrazolone and derivatives, 564
— — —, staphylococcal; an animal model for, Supp. 8, 28
— — — (the scalded skin syndrome); studies of staphylococcal toxins in relation to, Supp. 8, 35

Toxin, staphylococcal epidemolytic; action of on mouse skin in organ culture, Supp. 8, 40
—s, —; studies of, in relation to toxic epidermal necrolysis (the scalded skin syndrome), Supp. 8, 35
Treatment: antimetabolites in psoriasis, 87
  BAY b 5097 in fungal infections, 628
  combined cytostatic and surgical treatment in extensive basal cell epitheliomata, 408
  dapsone in herpetiform pemphigus foliaceus (case), 99
  ethambutol in tuberculosis, 211
  fluocinonide in psoriasis and eczema (letter), 310
  gluten-free diet in dermatitis herpetiformis (case), 200
  Grenz rays in recurrent herpes simplex, 172
  hydroxyurea in psoriasis (letter), 102
  hyperbaric oxygen therapy in dermatology, 631
  iontophoresis of tap water and poldine methosulphate in idiopathic hyperhidrosis, 72
  ointments in ichthyosis; double-blind trial of, 291
  propantheline, topical, in hyperhidrosis (letter), 441
  retinoic acid in naevus comedonicus, 528
  — — in psoriasis, 524
  rifampicin in tuberculosis, 210
  sodium bicarbonate in porphyria cutanea tarda (case), 200
  sulphur in acne vulgaris—is it helpful or harmful?, 620
  tetraynoic acid, topical, in the seborrhoea of acne, 66
  thioacetazone in tuberculosis, 212
  triamcinolone acetonide solution (by means of a needleless injector) in psoriatic nail dystrophy, 79
  4, 5′, 8-trimethylpsoralen (Trisoralen), oral, in vitiligo, 54
  turkey diet regime in psoriasis (letter), 440
4,5′, 8-Trimethylpsoralen (Trisoralen); treatment of vitiligo with, 54
Tuberculosis; " reserve " drugs in the treatment of, 210
Tumour, Jadassohn; arising at smallpox vaccination site, 177
Turkey diet regime in the treatment of psoriasis (letter), 440
Tyrosinase inhibitor; intracellular localization of in amelanotic and melanotic malignant melanoma, 385

Viomycin; cutaneous reactions to, 653
Vitiligo; treatment of with oral 4,5′, 8-trimethylpsoralen (Trisoralen), 54
Voigt's lines in negro children from the West Indies, 260
Vulva, diseases of: a review of the recent literature on—Part I, general aspect, 641

Wen; lipid synthesis (^{14}-acetate incorporation) in, cf. that in isolated human sebaceous gland, appendage-freed epidermis and sebocyst, 614

Zinc and copper in psoriasis (letter), 205, 439
— oxide; action of on dithranol (letter), 203

AUTHORS

Abell, E., treatment of psoriatic nail dystrophy, 79

Acharya, U.; McKenzie, A. W. and: the oral contraceptive and variegate porphyria, 453

Alexander, J. O'D., Dapsone (letter), 312

Alexander, Suzanne, "lymphocyte transformation" (letter), 103

Almeyda, J. and Levantine, A., Drug Reactions XVII, 313; XVIII, 651

Arbuthnott, J. P., Kent, Janet, Lyell, A. and Gemmell, C. G., studies of staphylococcal toxins in relation to toxic epidermal necrolysis (the scalded skin syndrome), Supp. 8, 35

Baird, J. Eileen; Dick, Heather M. and: an animal model for staphylococcal toxic epidermal necrolysis, Supp. 8, 28

Baker, H., Sattar, H.; Grice, Katherine and: treatment of idiopathic hyperhidrosis with iontophoresis of tap water and poldine methosulphate, 72

Barr, P.-O., Enfors, W. and Eriksson, G., hyperbaric oxygen therapy in dermatology, 631

Bartsokas, St. K., Capetanakis, J.; Stratigos, J. D. and: further experiences of toxic epidermal necrolysis incriminating allopurinol, pyrazolone and derivatives, 564

Bassett, D. C. J., streptococcal pyoderma and acute nephritis in Trinidad, Supp. 8, 55

Bauer, F. W., Thiele, F. A. J.; Hemels, H. G. W. M. and: effects of propranolol on the acetylcholine induced response of the human sweat gland (letter), 206

Bayles, Margaret A. H., coiffure keloids, 415

Bell, Mary and Montagna, W., innervation of sweat glands in horses and dogs, 160

Beutner, E. H.; Chorzelski, T. P. and: factors contributing to occasional failures in the laboratory diagnosis of bullous pemphigoid by indirect immunofluorescence, 111

Bewsher, P. D.; Stankler, L. and: chronic mucocutaneous candidiasis, endocrine deficiency and alopecia areata, 238

Bhattacharaya, M.; Carruthers, C. and: correlation between various proteins of bovine snout epidermis, 495

Binnie, W. H. and Cawson, R. A., a new ultrastructural finding in oral submucous fibrosis, 286

Black, M. M., the pathogenesis of lichen planus, 302

— and Wilson Jones, E., "lymphomatoid" pityriasis lichenoides; a variant with histological features simulating a lymphoma, 329

—, —; Marks, R. and: pityriasis lichenoides: a reappraisal, 215

Bleehen, S. S., treatment of vitiligo with oral 4,5′8-trimethylpsoralen (Trisoralen), 54

Bottoms, Eva, Wyatt, E. and Comaish, S., progressive changes in cuticular pattern along the shafts of human hair as seen by scanning electron microscopy, 379

Bradley, W. G.; Williams, M. H. and: an assessment of dapsone toxicity in the guinea-pig (letter), 650

Brooks, S. E. H., James, W. P. T., Louisy, Sheilah; Warner, Joyce and: juvenile dermatitis herpetiformis in Jamaica: clinical and gastrointestinal features, 226

Brorson, J.-E., Roupe, G. and Seeberg, S., tetracycline-resistant β-haemolytic streptococci in dermatological patients, 449

Bullough, W. S. and Deol, Johanna U. R., chalone control of mitotic activity in eccrine sweat glands, 586

Burrows, D., dermatitis herpetiformis; improvement with gluten-free diet (case), 200

—, porphyria cutanea tarda treated with sodium bicarbonate (case), 200

—, the prevalence of dermatitis herpetiformis (letter), 437

Burton, J. L. and Shuster, S., the effect of topical tetraynoic acid on the seborrhoea of acne, 66

—, —; Cartlidge, M. and: the effect of prolonged topical application of an anticholinergic agent on the sebaceous glands, 61

Campbell, C.; Kurwa, A. and: *Hendersonula toruloidea* infection (case), 98

Capetanakis, J., Bartsokas, St. K.; Stratigos, J. D. and: further experiences of toxic epidermal necrolysis incriminating allopurinol, pyrazolone and derivatives, 564.

Carruthers, C. and Bhattacharaya, M., correlation between various proteins of bovine snout epidermis, 495

Cartlidge, M., Burton, J. L. and Shuster, S., the effect of prolonged topical application of an anticholinergic agent on the sebaceous glands, 61

Cawson, R. A.; Binnie, W. H. and: a new ultrastructural finding in oral submucous fibrosis, 286

Cerimele, D., Serri, F.; Yamasawa, S. and: the activity of metabolic enzymes of human epidermis in relation to age, 134

Chorzelski, T. P. and Beutner, E. H., factors contributing to occasional failures in the laboratory diagnosis of bullous pemphigoid by indirect immunofluorescence, 111

Clayton, Yvonne M.; Midgley, Gillian and: distribution of dermatophytes and Candida spores in the environment, Supp. 8, 69

Comaish, S., white scalp hairs turning black—an unusual reversal of the ageing process, 513

— and Smith, J., action of zinc oxide on dithranol (letter), 203

—, Wyatt, E.; Bottoms, Eva and: progressive changes in cuticular pattern along the shafts of human hair as seen by scanning electron microscopy, 379

Connor, B., herpetiform pemphigus foliaceus responsive to dapsone (case), 99

—, impetigo contagiosa in the United Kingdom, Supp. 8, 48

—, mycosis fungoides with dermatomyositis (case title), 101

Constable, Hilary, ultrastructure of adult epidermal cells in monolayer culture, 27

Cooper, M. and Mishima, Y., increased *in vitro* radio-sensitivity of malignant melanoma induced by the *in vivo* administration of chlorpromazine, 491

Cotterill, J. A., Shakespeare on the skin, 533

—, Cunliffe, W. J. and Williamson, B., variations in skin surface lipid composition and sebum excretion rate with different sampling techniques, II, 356

—, Williamson, B.; Cunliffe, W. J. and: the effect of a medicated wash on acne, sebum excretion rate and skin surface lipid composition (letter), 311

—, —, Forster, R. A.; Cunliffe, W. J. and: the relevance of skin surface lipids to acne vulgaris, Supp. 8, 10

Cotton, D. W. K. and Van den Hurk, J. J. M. A., fluorimetric assay of carboxylic ester hydrolases in guinea-pig skin and the effects of propranolol, 608

Cronin, E., Contact Dermatitis XVII, 104

Cunliffe, W. J., Cotterill, J. A. and Williamson, B., the effect of a medicated wash on acne, sebum excretion rate and skin surface lipid composition (letter), 311

—, —, Williamson, B. and Forster, R. A., the relevance of skin surface lipids to acne vulgaris, Supp. 8, 10

—, Johnson, C. E. and Williamson, D. M., localized unilateral hyperhidrosis—a clinical and laboratory study, 374

—, Williamson, B.; Cotterill, J. A. and: variations in skin surface lipid composition and sebum excretion rate with different sampling techniques, II, 356

Dave, V. K., eccrine sweat gland carcinoma with metastases, 95

Dawber, R. P. R., Marks, R. and Swift, J. A., scanning electron microscopy of the stratum corneum, 272

Decherd, J. W., Mills, O. and Leyden, J. J., naevus comedonicus—treatment with retinoic acid, 528

—, Schlappner, O. L. A., Heaton, C. L.; Stein, K. M. and: demonstration of basal cell immunofluorescence in drug-induced toxic epidermal necrolysis, 246

Deol, Johanna U. R.; Bullough, W. S. and: chalone control of mitotic activity in eccrine sweat glands, 586

DeWit, Sharon, Ferrington, R. A.; Lewin, K. and: pathology of the finger nail in psoriasis, 555

Dick, Heather M. and Baird, J. Eileen, an animal model for staphylococcal toxic epidermal necrolysis, Supp. 8, 28

Dickson, A., Macleod, T. M., Frain-Bell, W.; Gardiner, J. S. and: the investigation of photocontact dermatitis in a dye manufacturing process, 264

Dixon, P. N., Warin, R. P. and English, Mary P., alimentary *Candida albicans* and napkin rashes, 458

—, —, —, *Candida albicans* infection and napkin rash (letter), 207

Dover, J. R.; Robinson, T. W. E. and: experimental zosteriform herpes simplex virus infection in mouse skin, 40

Dubinin, I., Smetana, K., Hrdlička, A., Horňák, O., Ohelert, W.; Karásek, J. and: nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte maturation, 601

Dupuis, G., Towers, G. H. N.; Mitchell, J. C. and: allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp.). The roles of pyrethrosin, a sesquiterpene lactone, and of pyrethrin II, 568

Earle, J.; Porter, D. and: Jadassohn tumour arising at smallpox vaccination site, 177

English, Mary P., epidemiology of animal ringworm in man, Supp. 8, 78

—, Warin, R. P.; Dixon, P. N. and: alimentary *Candida albicans* and napkin rashes, 458

—, —; —: *Candida albicans* infection and napkin rash (letter), 207

—, —; O'Grady, K. J. and: *Microsporum equinum* infection of the scalp in an adult, 175

Enfors, W., Eriksson, G.; Barr, P.-O. and: hyperbaric oxygen therapy in dermatology, 631

Eriksson, G., Enfors. W.; Barr, P.-O. and: hyperbaric oxygen therapy in dermatology, 631

Evans, C. D., a history of dermatology in Bristol and the West of England, 180

Feinstein, A.; Ziprkowski, L. and: a survey of ichthyosis vulgaris in Israel, 1

Ferrington, R. A., DeWit, Sharon; Lewin, K. and: pathology of the finger nail in psoriasis, 555

Feuerman, E. and Levy, A., a study of the effect of prednisone and an antihistamine on patch test reactions, 68

Fisher, L. B. and Maibach, H. I., the effect of occlusive and semipermeable dressings on the mitotic activity of normal and wounded human epidermis, 593

Forster, R. A., Cotterill, J. A,. Williamson, B.; Cunliffe, W. J. and: the relevance of skin surface lipids to acne vulgaris, Supp. 8, 10

Frain-Bell, W., Dickson, A., Macleod, T. M.; Gardiner, J. S. and: the investigation of photocontact dermatitis in a dye manufacturing process, 264

Frankland, J. C.; Seville, R. H. and: treatment of hyperhidrosis with topical propantheline—a new technique (letter), 441

Fredriksson, T., topical treatment with BAY b 5097, a new broad spectrum antimycotic agent, 628

Frenk, E., Smith, A. J.; Walker, Anne E. and: Therapeutics XVII, 210

Fry, L.; MacDonald, A. and: retinoic acid in the treatment of psoriasis, 524

Gardiner, J. S., Dickson, A., Macleod, T. M., and Frain-Bell, W., the investigation of photocontact dermatitis in a dye manufacturing process, 264

Gaylarde, P., Macmillan, A. L. and Sarkany, I., penetration and dose of injections with the Porton jet injector, 83

Gemmell, C. G., Kent, Janet, Lyell, A.; Arbuthnott, J. P. and: studies of staphylococcal toxins in relation to toxic epidermal necrolysis (the scalded skin syndrome), Supp. 8, 35

Goolamali, S. K. and Stevenson, C. J., granuloma annulare in identical twins, 636

Grasso, P.; Lansdown, A. B. G., and: physico-chemical factors influencing epidermal damage by surface active agents: 361

Greaves, M. W., zinc and copper in psoriasis (letter), 439

Grice, Katherine, Sattar, H. and Baker, H., treatment of idiopathic hyperhidrosis with iontophoresis of tap water and poldine methosulphate, 72

Günther, W.; Klug, H. and: ultrastructural differences in human malignant melanomata, 395

Hall, J., Haroon, A.; Haroon, T. S. and: effect of topical tetraynoic acid on sebum excretion, 64

Halliwell, R. E. W., Schwartzman, R. M.; Kelly, D. F. and: generalized cutaneous eruption in a dog, with histological similarity to human mycosis fungoides, 164

Halprin, K. M., epidermal " turnover time " —a re-examination, 14

Hamada, T. and Mishima, Y., intracellular localization of tyrosinase inhibitor in amelanotic and melanotic malignant melanoma, 385

Haroon, T. W., Hall, J. and Haroon, A., effect of topical tetraynoic acid on sebum excretion, 64

Heaton, C. L., Schlappner, O. L. A., Decherd, J. W.; Stein, K. M. and: demonstration of basal cell immunofluorescence in drug-induced toxic epidermal necrolysis, 246

Heiss, H. B., Schlappner, O. L. A.; Shelley, W. B. and: demonstration of intercellular immunofluorescence and epidermal hysteresis in bullous fixed drug eruption due to phenolphthalein, 118

Hell, Elizabeth, and Maibach, H., a comparison of *in vivo* and *in vitro* methods of identifying human epidermal cells in DNA synthesis, 506

Hellier, F. F., the prevalence of dermatitis herpetiformis (letter), 437

Hemels, H. G. W. M., Thiele, F. A. J. and Bauer, F. W., effects of propranolol on the acetylcholine induced response of the human sweat gland (letter), 206

Higgs, Janet M. and Wells, R. S., chronic mucocutaneous candidiasis: associated abnormalities of iron metabolism, Supp. 8, 88

Holla, S. W. J., Hollman, E. P. M. J., Mier, P. D., v.d. Staak, W. J. B. M., Urselmann, E. and Warndorff, J. A., adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin. II. Levels in atopic dermatitis, 147

Holland, P. and White, R. G., cutaneous reactions produced by *o*-chlorobenzylidenemalononitrile and *ω*-chloroacetophenone when applied directly to the skin of human subjects, 150

Hollman, E. P. M. J., Mier, P. D., v.d. Staak, W. J. B. M., Urselmann, E., Warndorff, J. A.; Holla, S. W. J. and: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin. II. Levels in atopic dermatitis, 147

Holt, R. J., the pathogenic role of coagulase-negative staphylococci, Supp. 8, 42

Honeyman, J. F., Winkler, N. W.; Lobitz, W. C., Jr. and: suppressed cell-mediated immunity in two adults with atopic dermatitis, 317

Hor^{N}ák, O., Smetana, K., Hrdlička, A., Dubinin, I., Ohelert, W.; Karásek, J. and: nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte maturation, 601

Hrdlička, A., Smetana, K., Dubinin, I., Horňák, O., Ohelert, W.; Karásek, J. and: nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte, maturation, 601

James, W. P. T., Brooks, S. E. H., Louisy, Sheilah; Warner, Joyce and: juvenile dermatitis herpetiformis in Jamaica: clinical and gastrointestinal features, 226

Jarrett, A. and Van Wyk, W., the quantitation of keratinization in human foetal tissues, 508

Johnson, C. E., Williamson, D. M.; Cunliffe, W. J. and: localized unilateral hyperhidrosis—a clinical and laboratory study, 374

Jordan, J. M. and Whitlock, F. A., emotions and the skin: the conditioning of scratch responses in cases of atopic dermatitis, 574

Kanan, M. W., cement dermatitis and atmospheric parameters in Kuwait, 155

Karásek, J., Smetana, K., Hrdlička, A., Dubinin, I., Horňák, O. and Ohelert, W., nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte maturation, 601

Kelly, Agnese, turkey diet regime in the treatment of psoriasis (letter), 440

Kelly, D. F., Halliwell, R. E. W. and Schwartzman, R. M., generalized cutaneous eruption in a dog, with histological similarity to human mycosis fungoides, 164

Kent, Janet, Lyell, A., Gemmell, C. G.; Arbuthnott, J. P. and: studies of staphylococcal toxins in relation to toxic epidermal necrolysis (the scalded skin syndrome), Supp. 8, 35

Kligman, A. M.; Mills, O. H., Jr. and: is sulphur helpful or harmful in acne vulgaris?, 620

Klug, H. and Günther, W., ultrastructural differences in human malignant melanomata, 395

Knight, A. G., Grenz ray treatment of recurrent herpes simplex, 172

Kurwa, A. and Campbell, C., *Hendersonula toruloidea* infection (case), 98

Lansdown, A. B. G. and Grasso, P., physicochemical factors influencing epidermal damage by surface active agents, 361

Lazarus, G. S., collagen, collagenase and clinicians, 193

Levantine, A.; Almeyda, J. and: Drug Reactions XVII, 313; XVIII, 651

Levene, G. M., immunology of streptococcal infection, Supp. 8, 62

Levine, M., the growth of adult human skin *in vitro*, 481

Levy, A.; Feuerman, E. and: a study of the effect of prednisone and an antihistamine on patch test reactions, 68

Lewin, K., DeWit, Sharon and Ferrington, R. A., pathology of the finger nail in psoriasis, 555

Lewis, K. G. S., Rees, J. K., Wells, R. S.; Pope, F. M. and: out-patient treatment of ichthyosis: a double-blind trial of ointments, 291

Leyden, J. J., Mills, O.; Decherd, J. W. and: naevus comedonicus—treatment with retinoic acid, 528

Lobitz, W. C., Jr., Honeyman, J. F. and Winkler, N. W., suppressed cell-mediated immunity in two adults with atopic dermatitis, 317

Louisy, Sheilah, Brooks, S. E. H., James, W. P. T.; Warner, Joyce and: juvenile dermatitis herpetiformis in Jamaica: clinical and gastrointestinal features, 226

Lyell, A., Kent, Janet, Gemmell, C. G.; Arbuthnott, J. P. and: studies of staphylococcal toxins in relation to toxic epidermal necrolysis (the scalded skin syndrome), Supp. 8, 35

McCallum, H. Morag, action of staphylococcal epidemolytic toxin on mouse skin in organ culture, Supp. 8, 40

Macdonald, A. and Fry, L., retinoic acid in the treatment of psoriasis, 524

McKenzie, A. W. and Acharya, U., the oral contraceptive and variegate porphyria, 453
Macleod, T. M., Dickson, A., Frain-Bell, W.; Gardiner, J. S. and: the investigation of photocontact dermatitis in a dye manufacturing process, 264
Macmillan, A. L., Sarkany, I.; Gaylarde, P. and: penetration and dose of injections with the Porton jet injector, 83
Maibach, H. I.; Fisher, L. B. and: the effect of occlusive and semipermeable dressings on the mitotic activity of normal and wounded human epidermis, 593
—; Hell, Elizabeth and: a comparison of *in vivo* and *in vitro* methods of identifying human epidermal cells in DNA synthesis, 506
Marks, R., histochemical applications of skin surface biopsy, 20
—, the role of chalones in epidermal homeostasis, 543
—, Black, M. and Wilson Jones, E., pityriasis lichenoides: a reappraisal, 215
—, Swift, J. A.; Dawber, R. P. R. and: scanning electron microscopy of the stratum corneum, 272
Meinert, W.; Weber, G. and: combined cytostatic and surgical treatment of extensive basal cell epitheliomata, 408
Midgley, Gillian and Clayton, Yvonne M., distribution of dermatophytes and Candida spores in the environment, Supp. 8, 69
Mier, P. D., Hollman, E. P. M. J., v.d. Staak, W. J. B. M., Urselmann, E., Warndorff, J. A.; Holla, S. W. J. and: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin. II. Levels in atopic dermatitis, 147
— and Sutorius, A. H. M., the control of glycogen utilization in skin; phosphorylase B kinase and phosphorylase A phosphatase, 49
— and Urselmann, E., adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin, 141
Miller, Ann, Munro, D. D.; Verbov, J. and: recurrent eczema herpeticum associated with ichthyosis vulgaris, 638
Mills, O., Leyden, J. J.; Decherd, J. W. and: naevus comedonicus—treatment with retinoic acid, 528
— and Kligman, A. M., is sulphur helpful or harmful in acne vulgaris?, 620
Mishima, Y.; Cooper, M. and: increased *in vitro* radio-sensitivity of malignant melanoma induced by the *in vivo* administration of chlorpromazine, 491
—; Hamada, T. and: intracellular localization of tyrosinase inhibitor in amelanotic and melanotic malignant melanoma, 385
Mitchell, J. C., Dupuis, G. and Towers, G. H. N., allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp.). The roles of pyrethrosin, a sesquiterpene lactone, and of pyrethrin II, 568
Molin, L.; Nyström, B. and: colonization with staphylococci in dermatology wards with different designs and practices, Supp. 8, 21
Molokhia, M. M.; Portnoy, B. and: zinc and copper in psoriasis (letter), 205
Montagna, W.; Bell, Mary, and: innervation of sweat glands in horses and dogs, 160
— and Yun, J. S., glands of Montgomery, 126
Munro, D. D., Miller, Ann; Verbov, J. and: recurrent eczema herpeticum associated with ichthyosis vulgaris, 638

Newbold, P. C. H., antimetabolites and psoriasis, 87
—, pre-cancer and the skin, 417
Nyström, B. and Molin, L., colonization with staphylococci in dermatology wards with different designs and practices, Supp. 8, 21

O'Grady, K. J., English, M. P. and Warin, R. P., *Microsporum equinum* infection of the scalp in an adult, 175
Ohelert, W., Smetana, K., Hrdlička, A., Dubinin, I., Horňák, O.; Karásek, J. and: nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte maturation, 601

Pegum, J. S., scleredema of Buschke (case title), 101
Poirier, J., Touraine, R.; Revuz, J. and: macular amyloidosis cutis (letter), 203
Pope, F. M., Rees, J. K., Wells, R. S. and Lewis, K. G. S., out-patient treatment of ichthyosis: a double-blind trial of ointments, 291
Porter, D. and Earle, J., Jadassohn tumour arising at smallpox vaccination site, 177
Portnoy, B. and Molokhia, M. M., zinc and copper in psoriasis (letter), 205
— and Sarkany, I., " Metosyn " in psoriasis and eczema (letter,) 310

Rasmussen, J. E., disseminated elastosis perforans serpiginosa in four mongoloids, 9
Rees, J. K., Wells, R. S., Lewis, K. G. S.; Pope, F. M. and: out-patient treatment of ichthyosis: a double-blind trial of ointments, 291

Revuz, J. Poirier, J. and Touraine, R., macular amyloidosis cutis (letter), 203

—, Tulliez, M.; Touraine, R. and: psoriasis and hydroxyurea (letter), 102

Ridley, C. M., a review of the recent literature on diseases of the vulva—Part I, general aspect, 641

Robinson, T. W. E. and Dover, J. R., experimental zosteriform herpes simplex virus infection in mouse skin, 40

Rodrigo, F. G., ultrastructural aspects of dermatitis herpetiformis (Duhring's disease), 348

Roupe, G., Seeberg, S.; Brorson, J.-E. and: tetracycline-resistant β-haemolytic streptococci in dermatological patients, 449

Ryan, S. F., Young, A. W., Jr.; Wagers, L. T. and: linear melorheostotic scleroderma, 297

Saikia, N. K., extraction of pemphigus antibodies from a lymphoid neoplasm and its possible relationship to pemphigus vulgaris, 411

Sarkany, I., malignant melanomata in lymphoedematous arm following radical mastectomy for breast carcinoma (an extension of the syndrome of Stewart and Treves) (case title), 101

—, Macmillan, A. L.; Gaylarde, P. and: penetration and dose of injections with the Porton jet injector, 83

—; Portnoy, B. and: "Metosyn" in psoriasis and eczema (letter), 310

Sattar, H., Baker, H.; Grice, Katherine and: treatment of idiopathic hyperhidrosis with iontophoresis of tap water and poldine methosulphate, 72

Savage, Doreen; Stewart, T. W. and: cosmetic camouflage in dermatology, 530

Savin, J. A., the bacteriology of acne, Supp. 8, 3

Schlappner, O. L. A., Heaton, C. L. Decherd, J. W.; Stein, K. M. and: demonstration of basal cell immunofluorescence in drug-induced toxic epidermal necrolysis, 246

—, Heiss, H. B.; Shelley, W. B. and: demonstration of intercellular immunofluorescence and epidermal hysteresis in bullous fixed drug eruption due to phenolphthalein, 118

Schwartzman, R. M., Halliwell, R. E. W.; Kelly, D. F. and: generalized cutaneous eruption in a dog, with histological similarity to human mycosis fungoides, 164

Searle, C. E., the selective depigmenting action of 8-hydroxyquinoline on hair growth in the mouse, 472

Seeberg, S., Roupe, G.; Brorson, J.-E. and: tetracycline-resistant β-haemolytic streptococci in dermatological patients, 449

Serri, F., Cerimele, D.; Yamasawa, S. and: the activity of metabolic enzymes of human epidermis in relation to age, 134

Seville, R. H. and Frankland, J. C., treatment of hyperhidrosis with topical propantheline—a new technique (letter), 441

Shelley, W. B., Schlappner, O. L. A. and Heiss, H. B., demonstration of intercellular immunofluorescence and epidermal hysteresis in bullous fixed drug eruption due to pheolphthalein, 118

Shuster, S.; Burton, J. L. and: the effect of topical tetraynoic acid on the seborrhoea of acne, 66

—, —; Cartlidge, M. and: the effect of prolonged topical application of an anticholinergic agent on the sebaceous glands, 61

Smetana, K., Hrdlička, A., Dubinin, I., Horňák, O., Ohelert, W.; Karásek, J. and: nuclear and nucleolar ultrastructure during the late stages of normal human keratinocyte maturation, 601

Smith, A. J., Frenk, E.; Walker, Anne E. and: Therapeutics XVII, 210

Smith, J.; Comaish, S. and: action of zinc oxide on dithranol (letter), 203

Somerville, Dorothy A., the microbiology of the cutaneous diphtheroids, Supp. 8, 16

Staak, W. J. B. M. v.d., Hollman, E. P. M. J., Mier, P. D., Urselmann, E., Warndorff, J. A.; Holla, S. W. J. and: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin. II. Levels in atopic dermatitis, 147

Stankler, L. and Bewsher, P. D., chronic mucocutaneous candidiasis, endocrine deficiency and alopecia areata, 238

Stein, K. M., Schlappner, O. L. A., Heaton, C. L. and Decherd, J. W., demonstration of basal cell immunofluorescence in drug-induced toxic epidermal necrolysis, 246

Stevanović, D. V., congenital diffuse mottling of the skin, 515

Stevens, J. C.; Wyatt, E. H. and: dapsone induced peripheral neuropathy, 521

Stevenson, C. J.; Goolamali, S. K. and: granuloma annulare in identical twins, 636

Stewart, T. W. and Savage, Doreen, cosmetic camouflage in dermatology, 530

Stratigos, J. D., Bartsokas, St. K. and Capetanakis, J., further experiences of toxic epidermal necrolysis incriminating allopurinol, pyrazolone and derivatives, 564

Summerly, R. and Woodbury, Sheila, lipid synthesis (^{14}-acetate incorporation) in the isolated human sebaceous gland, the appendage-freed epidermis, the sebocyst (steatocystoma) and the wen (keratinous cyst of skin), 614

Sutorius, A. H. M.; Mier, P. D. and: the control of glycogen utilization in skin; phosphorylase B kinase and phosphorylase A phosphatase, 49

Swift, J. A., Marks, R.; Dawber, R. P. R. and: scanning electron microscopy of the stratum corneum, 272

Thiele, F. A. J., Bauer, F. W.; Hemels, H. G. W. M. and: effects of propranolol on the acetylcholine induced response of the human sweat gland (letter), 206

— and Van Kempen, L. H. J., a micro method for measuring the carbon dioxide release by small skin areas, 463

Touraine, R., Poirier, J.; Revuz, J. and: macular amyloidosis cutis (letter), 203

—, Revuz, J. and Tulliez, M., psoriasis and hydroxyurea (letter), 102

Towers, G. H. N., Dupuis, G.; Mitchell, J. C. and: allergic contact dermatitis from pyrethrum (*Chrysanthemum* spp.). The roles of pyrethrosin, a sesquiterpene lactone, and of pyrethrin II, 568

Tulliez, M. Revuz, J.; Touraine, R. and: psoriasis and hydroxyurea (letter), 102

Urselmann, E., Hollman, E. P. M. J., Mier, P. D., v.d. Staak, W. J. B. M., Warndorff, J. A.; Holla, S. W. J. and: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin. II. Levels in atopic dermatitis, 147

—; Mier, P. D. and: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin, 141

Van den Hurk, J. J. M. A.; Cotton, D. W. K. and: fluorimetric assay of carboxylic ester hydrolases in guinea-pig skin and the effects of propranolol, 608

Van Kempen, L. H. J.; Thiele, F. A. J. and: a micro method for measuring the carbon dioxide release by small skin areas, 463

Van Wyk, W.; Jarrett, A. and: the quantitation of keratinization in human foetal tissues, 508

Verbov, J., acrokeratosis verruciformis of Hopf with steatocystoma multiplex and hypertrophic lichen planus, 91

—, fixed drug eruption due to phenazone in a hypnotic (letter), 438

—, Munro, D. D. and Miller, Ann, recurrent eczema herpeticum associated with ichthyosis vulgaris, 638

Vollum, Dorothy I., skin markings in negro children from the West Indies, 260

Wagers, L. T., Young, A. W., Jr., and Ryan, S. F., linear melorheostotic scleroderma, 297

Walker, Anne E., Frenk, E. and Smith, A. J., Therapeutics XVII, 210

Warin, R. P., English, Mary P.; Dixon, P. N. and: alimentary *Candida albicans* and napkin rashes, 458

—, —; —: *Candida albicans* infection and napkin rash (letter), 207

—, —; O'Grady, K. J. and: *Microsporum equinum* infection of the scalp in an adult, 175

Warndorff, J. A., the response of the sweat glands to β-adrenergic stimulation, 282

—, Hollman, E. P. M. J., Mier, P. D., v.d. Staak, W. J. B. M., Urselmann, E.; Holla, S. W. J. and: adenosine 3′ : 5′-cyclic monophosphate phosphodiesterase in skin. II. Levels in atopic dermatitis, 147

Warner, Joyce, Brooks, S. E. H., James, W. P. T. and Louisy, Sheilah, juvenile dermatitis herpetiformis in Jamaica: clinical and gastrointestinal features, 226

Weber, G., rosacea-like dermatitis: contraindication or intolerance reaction to strong steroids, 253

— and Meinert, W., combined cytostatic and surgical treatment of extensive basal cell epitheliomata, 408

Wells, R. S.; Higgs, Janet M. and: chronic mucocutaneous candidiasis: associated abnormalities of iron metabolism, Supp. 8, 88

—, Rees, J. K., Lewis, K. G. S.; Pope, F. M. and: out-patient treatment of ichthyosis: a double-blind trial of ointments, 291

White, R. G.; Holland, P. and: cutaneous reactions produced by *o*-chlorobenzylidenemalononitrile and ω-chloroacetophenone when applied directly to the skin of human subjects, 150

Whitlock, F. A.; Jordan, J. M. and: emotions and the skin: the conditioning of scratch responses in cases of atopic dermatitis, 574

Wilkinson, D. S., Contact Dermatitis XVIII, 445

Williams, M. H. and Bradley, W. G., an assessment of dapsone toxicity in the guinea-pig (letter), 650

Williamson, B., Cotterill, J. A.; Cunliffe, W. J. and: the effect of a medicated wash on acne, sebum excretion rate and skin surface lipid composition (letter), 311

—, —, FORSTER, R. A.; CUNLIFFE, W. J. and: the relevance of skin surface lipids to acne vulgaris, Supp. 8, 10

—, CUNLIFFE, W. J.; COTTERILL, J. A. and: variations in skin surface lipid composition and sebum excretion rate with different sampling techniques, II, 356

WILLIAMSON, D. M., JOHNSON, C. E.; CUNLIFFE, W. J. and: localized unilateral hyperhidrosis—a clinical and laboratory study, 374

WILSON JONES, E.; BLACK, M. M. and: " lymphomatoid " pityriasis lichenoides; a variant with histological features simulating a lymphoma, 329

—, —; MARKS, R. and: pityriasis lichenoides: a reappraisal, 215

WINKLER, N. W., HONEYMAN, J. F.; LOBITZ, W. C., Jr. and: suppressed cell-mediated immunity in two adults with atopic dermatitis, 317

WOODBURY, Sheila; SUMMERLY, R. and: lipid synthesis (^{14}-acetate incorporation) in the isolated human sebaceous gland, the appendage-freed epidermis, the sebocyst (steatocystoma) and the wen (keratinous cyst of skin), 614

WYATT, E., COMAISH, S.: BOTTOMS, Eva, and: progressive changes in cuticular pattern along the shafts of human hair as seen by scanning electron microscopy, 379

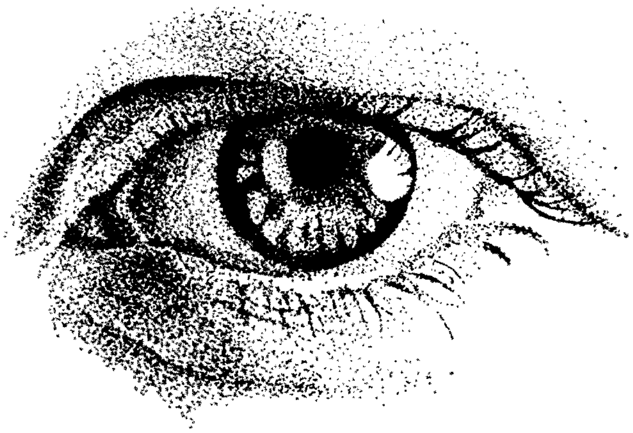
— and STEVENS, J. C., dapsone induced peripheral neuropathy, 521

YAMASAWA, S., CERIMELE, D. and SERRI, F., the activity of metabolic enzymes of human epidermis in relation to age, 134

YOUNG, A. W., Jr., RYAN, S. F.; WAGERS, L. T. and: linear melorheostotic scleroderma, 297

YUN, J. S.; MONTAGNA, W. and: glands of Montgomery, 126

ZIPRKOWSKI, L. and FEINSTEIN, A., a survey of ichthyosis vulgaris in Israel, 1



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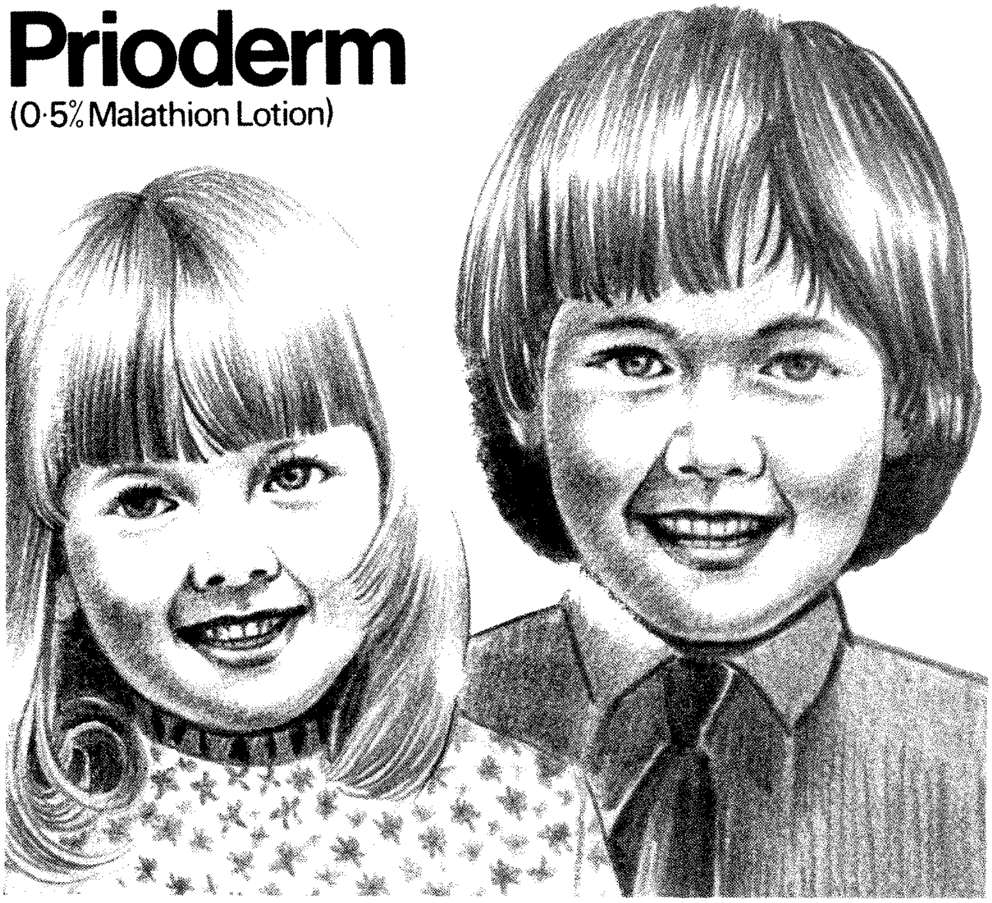

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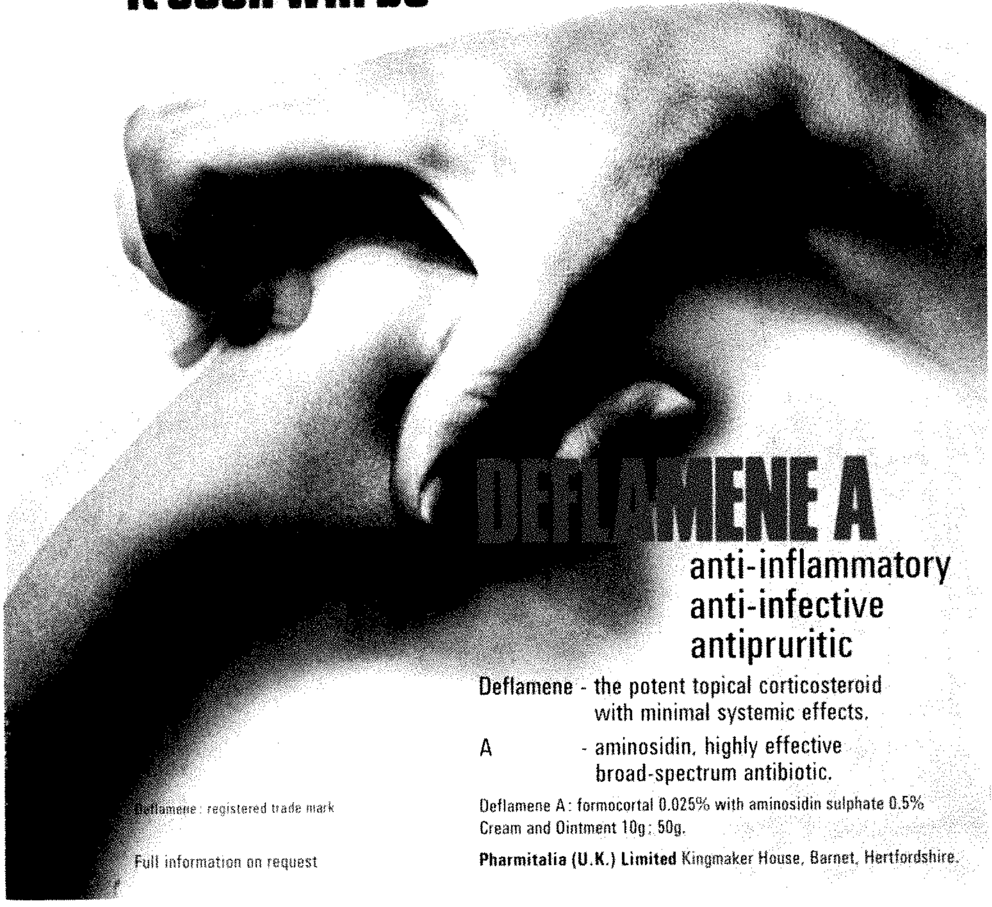

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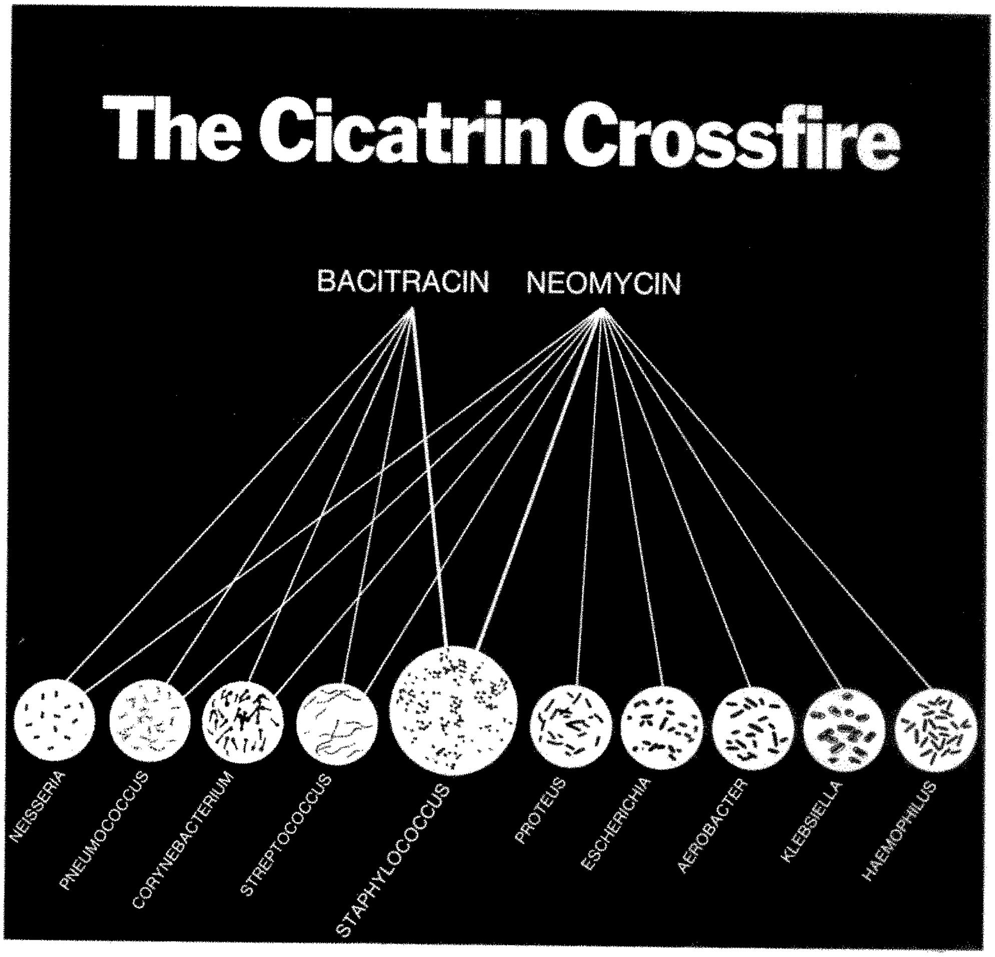

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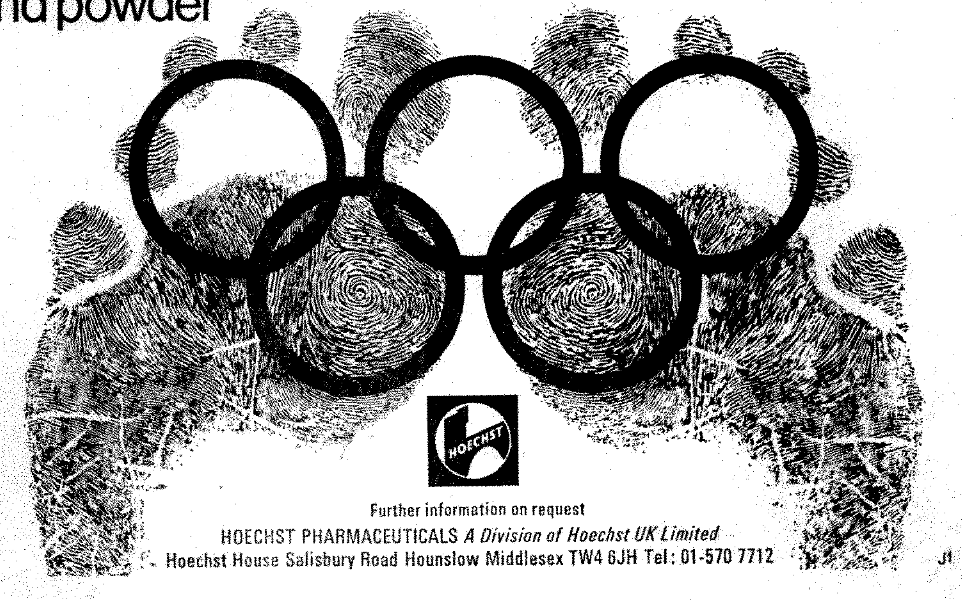

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CONTENTS (*continued*)

Review

A Review of the Recent Literature on Diseases of the Vulva—Part I General Aspects
C. M. Ridley 641

Books

Progress in Allergy. Vol. 15 (Kallos, Waksman and de Weck (Edit.)); Resistance to Infectious Disease (Dunlop and Moon (Edit.)); 648

Correspondence

An Assessment of Dapsone Toxicity in the Guinea-pig
M. H. Williams and W. G. Bradley 650

Quarterly Review

Drug Reactions XVIII. Cutaneous Reactions to Antituberculous Drugs
Ashley Levantine and John Almeyda 651

News and Notices 656

Index Volume 86 657

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

Pathology of the Finger Nail in Psoriasis. A Clinicopathological Study
KLAUS LEWIN, SHARON DEWIT and RICHARD A. FERRINGTON . 555
Further Experiences of Toxic Epidermal Necrolysis Incriminating Allopurinol, Pyrazolone and Derivatives
J. D. STRATIGOS, ST. K. BARTSOKAS and J. CAPETANAKIS . . 564
Allergic Contact Dermatitis from Pyrethrum (*Chrysanthemum* spp.) The Roles of Pyrethrosin, a Sesquiterpene Lactone, and of Pyrethrin II.
JOHN C. MITCHELL, GILLES DUPUIS and G. H. NEIL TOWERS . . 568
Emotions and the Skin: the Conditioning of Scratch Responses in Cases of Atopic Dermatitis
J. M. JORDAN and F. A. WHITLOCK 574
Chalone Control of Mitotic Activity in Eccrine Sweat Glands
W. S. BULLOUGH and JOHANNA U. R. DEOL 586
The Effect of Occlusive and Semipermeable Dressings on the Mitotic Activity of Normal and Wounded Human Epidermis
LOUIS B. FISHER and HOWARD I. MAIBACH 593
Nuclear and Nucleolar Ultrastructure during the Late Stages of Normal Human Keratinocyte Maturation
J. KARÁSEK, K. SMETANA, A. HRDLIČKA, I. DUBININ, O. HORŇÁK and W. OHELERT 601
Fluorimetric Assay of Carboxylic Ester Hydrolases in Guinea-pig Skin and the Effects of Propranolol
D. W. K. COTTON and JOSÉ J. M. A. VAN DEN HURK 608
Lipid Synthesis (^{14}C-Acetate Incorporation) in the Isolated Human Sebaceous Gland, the Appendage-freed Epidermis, the Sebocyst (Steatocystoma) and the Wen (Keratinous Cyst of Skin)
ROY SUMMERLY and SHEILA WOODBURY 614

Pharmacology and Treatment

Is Sulphur Helpful or Harmful in Acne Vulgaris?
OTTO H. MILLS, JR. and ALBERT M. KLIGMAN 620
Topical Treatment with Bay b 5097, a New Broad Spectrum Antimycotic Agent
TORSTEN FREDRIKSSON 628
Hyperbaric Oxygen Therapy in Dermatology
P.-O. BARR, WERA ENFORS and GUNNEL ERIKSSON 631

Case Reports

Granuloma Annulare in Identical Twins
S. K. GOOLAMALI and C. J. STEVENSON 636
Recurrent Eczema Herpeticum Associated with Ichthyosis Vulgaris
JULIAN VERBOV, D. D. MUNRO and ANN MILLER 638

(*Continued on* p. iii *of cover*)

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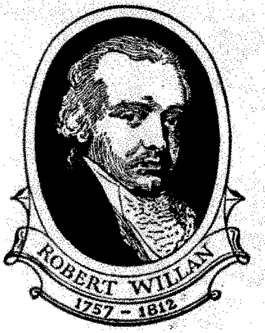

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CONTENTS

No. 1.—JULY

PAGE

Clinical and Laboratory Investigations

Senear-Usher Syndrome and Internal Malignancy. N. K. Saikia and L. E. S. MacConnell 1
Allergic Contact Dermatitis to Garlic. E. Bleumink, H. M. G. Doeglas, A. H. Klokke and J. P. Nater 6
The Effect of Enzyme Detergents on Transepidermal Water Loss from Normal Skin. Rowan Adams, C. L. Hales and E. J. Raffle 10
Human Epidermal Cell Cycle in Vitro. D. P. Chopra and B. A. Flaxman 13
Combined Surgical and Chemotherapeutic Treatment of Fortner's Amelanotic Hamster Melanoma AMel 3. E. Haneke, W. Wohlrab and J. Peker 18
The Adenosine Triphosphate Content of Guinea-pig Skin Stored at −196°C. S. A. Carney, M. Hall and C. R. Ricketts 23

Pharmacology and Treatment

The Effect of Glycerol on the Percutaneous Absorption of Methyl Nicotinate. Jonathan Hadgraft, J. W. Hadgraft and I. Sarkany . . 30
The Effect of Clindamycin in Acne—a Clinical and Laboratory Investigation. W. J. Cunliffe, J. A. Cotterill and B. Williamson 37
Treatment of Pityriasis Rubra Pilaris with Azathioprine. G. A. Hunter and I. J. Forbes 42

Case Reports

A Benign " Malignant " Ulcer. P. C. H. Newbold and R. B. Stoughton . 46
Unilateral Spider Naevi. W. J. Cunliffe, B. Dodman and M. J. Butterworth 51

Comment

Electron Microscopy: a Functional Approach. J. A. A. Hunter . . 53

Review

A Review of the Recent Literature on Diseases of the Vulva—Part II. Vulvitis: Infections. C. M. Ridley 58

Society Proceedings

Royal Society of Medicine, October 21st 1971 70

Obituary

Kenneth MacGregor 72

Correspondence

Psoriatic Alopecia. Sam Shuster 73
Pemphigus as an Autoimmune Disease. T. Chorzelski and S. Jabłońska . 78
Traction Alopecia due to " Hair Extension ". R. R. M. Harman . . 79
Innervation of Sweat Glands in Horses and Dogs. D. W. K. Cotton and P. van Hasselt 80

No. 1. JULY—*cont*

THE PATHOGENESIS OF LICHEN PLANUS. I. Sarkany and P. M. Gaylarde . 81
USE OF CIBA 1906 (THIAMBUTOSINE BP) IN VITILIGO. Julian Verbov . . 81

Books

CURRENT PROBLEMS IN DERMATOLOGY VOL. 4. (MALI (EDIT.)); MÉMENTO IMMUNOLOGIQUE DE LA SARCOIDOSE (CHRÉTIEN AND SALTIEL); THE PATHOLOGIC ANATOMY OF MYCOSES (BAKER (EDIT.)); CURRENT CONCEPTS OF THE HISTOLOGY OF ORAL MUCOSA (PROCEEDINGS OF SYMPOSIUM AT ILLINOIS) . 83

No. 2—AUGUST

Clinical and Laboratory Investigations

CUTANEOUS POLYARTERITIS NODOSA. Peter Borrie 87
CYTOLOGY OF THE MYCOSIS FUNGOIDES AND THE SÉZARY SYNDROME. CORRELATION BETWEEN LIGHT AND ELECTRON MICROSCOPY. J. Gomez Orbaneja, E. Sanchez Yus, L. Diaz-Flores and P. Simon Huarte 96
THE EPIDERMAL COMPONENT OF PITYRIASIS LICHENOIDES. R. Marks and M. M. Black 106
TOXOPLASMOSIS AND PITYRIASIS LICHENOIDES. N. B. Zlatkov and V. C. Andreev 114
THE ULTRASTRUCTURE OF LICHEN AMYLOIDOSUS WITH SPECIAL REFERENCE TO THE EPIDERMAL CHANGES. Martin M. Black and Christopher J. Heather 117
FAMILIAL LICHEN AMYLOIDOSIS. REPORT OF 19 CASES IN 4 GENERATIONS OF A CHINESE FAMILY IN MALAYSIA. K. Rajagopalan and C. H. Tay . . 123
A QUANTITATIVE STUDY OF ERYTHRASMA LESIONS. Dorothy A. Somerville . 130
EARLIEST FEATURES OF THE STRAWBERRY MARK IN THE NEWBORN. Akira Hidano and Shinobu Nakajima 138
PROGNOSIS IN INDUSTRIAL DERMATITIS. D. Burrows 145
THE EFFECT OF CHRONIC SUN DAMAGE ON THE ACTIVITY OF METABOLIC ENZYMES OF THE EPIDERMIS OF HUMAN SKIN. D. Cerimele, S. Yamasawa and F. Serri 149
CONDITIONING OF SWEATING. A PRELIMINARY REPORT. B. R. M. Kuypers and D. W. K. Cotton 154

Comment

PROSTAGLANDINS AND THE EPIDERMIS. Malcolm W. Greaves 161

Review

A REVIEW OF THE RECENT LITERATURE ON DISEASES OF THE VULVA—PART III. CARCINOGENESIS AND TUMOURS. C. M. Ridley 163

Society Proceedings

ROYAL SOCIETY OF MEDICINE, NOVEMBER 18TH, 1971 171
INTERNATIONAL SYMPOSIUM, ROME 1970 174

Correspondence

THE PHYSICAL PROPERTIES OF SKIN IN CUTIS LAXA. Rodney Grahame and Peter Beighton 176
THE GLANDS OF MONTGOMERY. F. O. C. Meenan 176
JEJUNAL BIOPSIES IN BULLOUS PEMPHIGOID. N. K. Saikia, A. McQueen and R. MacKie 177

No. 2. AUGUST—*cont*

Books

CUTANEOUS LESIONS OF THE LOWER EXTREMITIES (SAMITZ AND DANA); REGULATION OF MAST CELL FORMATION (CSABA (EDIT.)) 179

Quarterly Review

THERAPEUTICS XIX. LIPID LOWERING AGENTS. E. Frenk, Anne E. Walker and I. R. Edwards 180

News and Notices 185

No. 3.—SEPTEMBER

Clinical and Laboratory Investigations

THE CONTROL OF EPIDERMAL THICKNESS—PART I. William S. Bullough . 187
ELECTRONMICROSCOPIC STUDY OF HYPOPIGMENTED LESIONS IN LEPROSY. A PRELIMINARY REPORT. C. K. Job, Ammu Nayar and J. S. Narayanan . 200
THE ROLE OF THE CIRCULATION IN MEASUREMENTS OF SKIN CONDUCTIVITY. Ann Woolley-Hart 213
CHANGES IN THE FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS, TRIGLYCERIDES AND FREE FATTY ACIDS WITH DEPTH IN THE COW SNOUT EPIDERMIS. V. J. W. Long 227
ALLERGIC CONTACT DERMATITIS FROM SESQUITERPENOIDS OF PLANTS. ADDITIONAL ALLERGENIC SESQUITERPENE LACTONES AND IMMUNOLOGICAL SPECIFICITY OF COMPOSITAE, LIVERWORTS AND LICHENS. J. C. Mitchell, G. Dupuis and T. A. Geissman 235
SEBORRHOEIC DERMATITIS OF THE BEARD. John A. Parrish and Kenneth A. Arndt 241
DERMATITIS IN MAN AND IN A DOG CAUSED BY THE MITE *Cheyletiella yasguri* SMILEY. E. J. M. Bakkers and A. Fain 245

Pharmacology and Treatment

DEFICIENT FOLATE ACTIVITY DURING TREATMENT OF PSORIASIS WITH METHOTREXATE DIAGNOSED BY DETERMINATION OF SERINE SYNTHESIS IN LYMPHOCYTES. J. Ellegaard, V. Esmann and L. Henriksen 248
EFFECT OF RETINOIC ACID IN PSORIASIS, II. LONG TERM STUDY. Angus Macdonald, R. M. H. McMinn and Lionel Fry 256
STUDIES ON LOW DOSE CHLOROQUINE THERAPY AND THE ACTION OF CHLOROQUINE IN SYMPTOMATIC PORPHYRIA. J. J. F. Taljaard, B. C. Shanley, E. G. Stewart-Wynne, W. M. Deppe and S. M. Joubert 261
INTERMITTENT CHEMOTHERAPY IN THE TREATMENT OF TUBERCULOSIS CUTIS. A PRELIMINARY REPORT. V. S. Rajan and Y. S. Goh 270

Case Report

LOCALIZED ACNEIFORM ERUPTION FOLLOWING COBALT IRRADIATION. Kenneth M. Stein, James J. Leyden and Herbert Goldschmidt 274

Comment

THE NATURE, PATHOGENESIS AND STAINING PROPERTIES OF AMYLOID. Martin M. Black 280

No. 3.—SEPTEMBER—*cont.*

Obituaries

JOHN LEWIS FRANKLIN 284
E. C. RITTER 285
GILBERT HENLY 286
G. H. RITTERMAN 287

Society Proceedings

ROYAL SOCIETY OF MEDICINE, DECEMBER 16TH, 1971 288

Books

CONSULTATIONS IN DERMATOLOGY WITH WALTER B. SHELLEY; HAZARDS OF MEDICATION (MARTIN AND MARTIN (EDIT.)); THE THIRD CONFERENCE ON THE CLINICAL DELINEATION OF BIRTH DEFECTS, PART XII. SKIN, HAIR AND NAILS (BERGSMA (EDIT.)) 290

Correspondence

ZINC AND COPPER IN PSORIASIS. Benjamin Portnoy and Mohamed Molokhia . 291
THE EFFECT OF A MEDICATED WASH ON ACNE. F. Ray Bettley 292

Quarterly Review

DRUG REACTIONS XIX. ADVERSE CUTANEOUS REACTIONS TO THE PENICILLINS —AMPICILLIN RASHES. John Almeyda and A. Levantine 293

News and Notices 297

No. 4—OCTOBER

Clinical and Laboratory Investigations

HEREDITARY INFLAMMATORY VASCULITIS WITH PERSISTENT NODULES. A GENETICALLY-DETERMINED NEW ENTITY PROBABLY RELATED TO LUPUS ERYTHEMATOSUS. William B. Reed, Robert F. Bergeron, Denny Tuffanelli and Edward Wilson Jones 299
ATYPICAL ERYTHROKERATODERMA WITH DEAFNESS, PHYSICAL RETARDATION AND PERIPHERAL NEUROPATHY. J. Martin Beare, N. C. Nevin, P. Froggatt, D. C. Kernohan and Ingrid V. Allen 308
PERIORAL DERMATITIS: AETIOLOGY AND TREATMENT WITH TETRACYCLINE. Angus Macdonald and Michael Feiwel 315
NICKEL ANTIBODIES. Louis Forman and Suzanne Alexander 320
PILO-SEBACEOUS DUCT OBSTRUCTION AND ACNE. R. L. Holmes, M. Williams and W. J. Cunliffe 327
Age and Sex Variation in Skin Surface Lipid Composition and Sebum Excretion Rate. J. A. Cotterill, W. J. Cunliffe, B. Williamson and L. Bulusu . . 333
LICHEN PLANUS; AN INBORN ERROR OF METABOLISM. D. W. K. Cotton, José J. M. A. van den Hurk and W. B. J. M. van der Staak 341
THE CONTROL OF EPIDERMAL THICKNESS—PART 2. William S. Bullough . 347
ULTRASTRUCTURAL STUDY OF THE CUTANEOUS ELASTIC FIBRES IN LUPUS ERYTHEMATOSUS. D. Schmitt, J. Thivolet and H. Perrot 355
HAIR DIAMETER IN FEMALE BALDNESS. David Jackson, R. E. Church and F. J. Ebling 361

No. 4.—OCTOBER—*cont.*

ABNORMAL HAIR SHAFTS IN PSORIASIS ON SCANNING ELECTRON MICROSCOPY. Edward Wyatt, Eva Bottoms and Stanley Comaish 368

Case Report

POST KALA-AZAR DERMAL LEISHMANIASIS. D. D. Munro, Anthony du Vivier and W. H. Jopling 374

Pharmacology and Treatment

ULTRAVIOLET THERAPY OF PSORIASIS: A CRITICAL STUDY. E. Young . . 379

GRISEOFULVIN THERAPY IN LICHEN PLANUS. A DOUBLE-BLIND CONTROLLED TRIAL. V. N. Sehgal, G. J. S. Abraham and Gauri Bazaz Malik . . 383

Comment

RECENT PROGRESS IN THE FORMULATION OF VEHICLES FOR TOPICAL APPLICATIONS. J. W. Hadgraft 386

Obituary

ANDREW McCONNELL DAVIDSON 390

Books

DERMATOLOGY SPECIALTY BOARD REVIEW (SELMANOWITZ AND ORENTREICH); RADIUM RECIPES FOR CUTANEOUS CANCER (THE MANCHESTER METHOD) (HOWELL, MEREDITH AND SWINDELL) 391

Correspondence

PHOTOCONTACT DERMATITIS IN A DYE MANUFACTURING PROCESS. Peter S. Herman 392

RETINOIC ACID IN THE TREATMENT OF PSORIASIS. C. Howard Whittle . . 392

Society Proceedings

ROYAL SOCIETY OF MEDICINE, JANUARY 20TH, 1972 394

ROYAL SOCIETY OF MEDICINE, FEBRUARY 17TH, 1972 396

JOINT MEETING OF EUROPEAN SOCIETY FOR DERMATOLOGICAL RESEARCH AND THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY, MAY 17TH–19TH, 1972 . 398

Quarterly Review

CONTACT DERMATITIS XIX. SENSITIVITY TO CHROMIUM IN CEMENT DERMATITIS. NECROTIC REACTIONS TO APPLIED MEDICAMENTS. D. S. Wilkinson and Etain Cronin 400

News and Notices 402

No. 5.—NOVEMBER

Clinical and Laboratory Investigations

THE NATURAL HISTORY OF PARAPSORIASIS EN PLAQUES (CHRONIC SUPERFICIAL DERMATITIS) AND PRERETICULOTIC POIKILODERMA. Peter D. Samman . 405

PULMONARY EOSINOPHILIA ASSOCIATED WITH VASCULITIS AND EXTRA-VASCULAR GRANULOMATA. A CASE REPORT AND REVIEW OF THE LITERATURE. J. L. Burton and P. A. Burton 412

No. 5.—NOVEMBER—*cont.*

THE AETIOLOGY OF IMPETIGO CONTAGIOSA. M. el Zawahry, A. Abdel Aziz and M. Soliman 420
A ROSACEA-LIKE ERUPTION OF CHILDREN. J. A. Savin, Suzanne Alexander and R. Marks 425
PERIORAL DERMATITIS. Ian Sneddon 430
ULTRASTRUCTURAL FEATURES OF THE INVASIVE PHASE OF *Candida Albicans*. R. A. Cawson and K. C. Rajasingham 435
THE "WHEAT-STUBBLE SARCOID GRANULOMA": A NEW EPITHELIOID GRANULOMA OF THE SKIN. J. Cortez Pimentel 444
ACID HYDROLASES IN THE HUMAN EPIDERMIS. Akira Ohkawara, Kenneth M. Halprin, Joseph R. Taylor and Victor Levine 450
COMPARATIVE STUDY OF ^{3}H-THYMIDINE LABELLING OF THE DERMAL INFILTRATE OF SKIN ALLERGIC AND IRRITANT PATCH TEST REACTIONS IN MAN. J. M. Lachapelle 460
DIRECT IMMUNOFLUORESCENT STUDY OF THE SKIN ON OCCURRENCE OF COMPLEMENT IN PEMPHIGUS. Th. Van Joost, R. H. Cormane and K. W. Pondman 466
MEASUREMENT OF LIPID SYNTHESIS IN MOUSE AURICULAR SKIN CULTURES *in Vitro*. C. Prottey and T. F. M. Ferguson 475

Case Reports

UNUSUAL FEATURES OF SCABIES ASSOCIATED WITH TOPICAL FLUORINATED STEROIDS. A. L. Macmillan 496
ERYTHRODERMA DUE TO PERITRATE AND GLYCERYL TRINITRATE. F. P. Ryan 498
CROHN'S DISEASE OF THE MOUTH. L. Stankler, S. W. B. Ewen and N. W. Kerr 501
A CASE OF TINEA NIGRA IN BRITAIN. H. W. Chadfield and C. K. Campbell . 505

Comment

HAIR REPLACEMENT IN THE GUINEA-PIG AND IN MAN. David Jackson . . 509

Obituary

JOHN THORNTON INGRAM 1899–1972 512

Society Proceedings

IRISH DERMATOLOGICAL SOCIETY, APRIL 29TH, 1972 514

Books and Periodicals

PROTECTION AGAINST RADIATION FROM BRACHYTHERAPY SOURCES. RECOMMENDATIONS OF THE NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENTS; ARBEITSMEDIZIN, SOZIALMEDIZIN, ARBEITSHYGIENE; OCCUPATIONAL MYCOTIC INFECTIONS; AMERICAN ZOOLOGIST: SYMPOSIUM ON THE VERTEBRATE INTEGUMENT 515

Correspondence

SEPARATION OF STRATUM CORNEUM. A. Gafoor and K. M. S. Townsend . 516
ACTION OF STAPHYLOCOCCAL EPIDERMOLYTIC TOXIN ON MOUSE SKIN IN ORGAN CULTURE. I. Sarkany and P. M. Gaylarde 516

Quarterly Review

THERAPEUTICS XX. THE MANAGEMENT OF DEEP VENOUS THROMBOSIS. PART I. DIAGNOSIS. I. R. Edwards, Anne E. Walker and E. Frenk . 518

No. 5.—NOVEMBER—*cont*

News and Notices 522

No. 6.—DECEMBER

Clinical and Laboratory Investigations

CELL-MEDIATED IMMUNITY IN PATIENTS WITH MALIGNANT MELANOMA. R. M. Mackie, W. G. S. Spilg, C. E. Thomas and A. J. Cochran 523
PYODERMA GANGRENOSUM WITH ALTERED CELLULAR IMMUNITY AND DERMONECROTIC FACTOR. J. Delescluse, Cl. de Bast and G. Achten 529
THE INFLAMMATORY REACTION IN PITYRIASIS LICHENOIDES. M. M. Black and R. Marks 533
INSULIN PRODUCTION AND GLUCOSE TOLERANCE IN PATIENTS WITH GRANULOMA ANNULARE. Richard Hammond, Kirby Dyess and Albert Castro . . 540
CORTICOSTEROID-INDUCED ATROPHY OF THE SKIN WITH TELANGIECTASIA. A CLINICAL AND EXPERIMENTAL STUDY. Danilo V. Stevanović 548
NAEVUS LIPOMATOSUS CUTANEUS SUPERFICIALIS (HOFFMAN-ZURHELLE). A. G. Finley and L. A. Musso 557
GENERALIZED *Trichophyton Mentagrophytes* RINGWORM IN CAPUCHIN MONKEYS (*Cebus Nigrivitatus*). B. G. Bagnall and W. Grünberg 565
CYCLIC 3′, 5′ ADENOSINE MONOPHOSPHATE-DEPENDENT PROTEIN KINASE OF SKIN I. MEASUREMENT AND PROPERTIES. P. D. Mier and J. van den Hurk . 571
CYCLIC 3′, 5′ ADENOSINE MONOPHOSPHATE-DEPENDENT PROTEIN KINASE OF SKIN II. LEVELS IN ATOPIC DERMATITIS AND PSORIASIS. P. D. Mier and J. van den Hurk with S. W. J. Holla, E. P. M. J. Hollman, J. E. Porters and M. B. M. Weemers 577
EFFECTS OF CYTOCHALASIN B ON EPIDERMAL CELLS *in Vitro*. Hilary Constable, C. N. D. Cruickshank and Patricia R. Mann 580
LIPID SYNTHESIS IN RAT SKIN. C. Prottey, P. J. Hartop and T. F. M. Ferguson 586

Pharmacology and Treatment

THE EFFECT OF EICOSA 5 : 8 : 11 : 14 TETRAYNOIC ACID ON SKIN LIPID SYNTHESIS (^{14}C INCORPORATION) *in Vitro*. Roy Summerly, Sheila Woodbury and H. J. Yardley 608
PERCUTANEOUS ABSORPTION AND DISTRIBUTION OF 2-NAPHTHOL IN MAN. H. G. W. M. Hemels 614
STRUCTURAL AND FUNCTIONAL ABNORMALITIES OF THE LIVER IN PSORIASIS BEFORE AND DURING METHOTREXATE THERAPY. J. Almeyda, D. Barnardo, H. Baker, G. M. Levene and J. W. Landells 623
DOUBLE-BLIND TRIAL OF ZINC SULPHATE IN THE TREATMENT OF CHRONIC VENOUS LEG ULCERATION. M. W. Greaves and F. A. Ive 632

Case Reports

CONGENITAL MACULAR URTICARIA PIGMENTOSA COMPLICATED BY MASSIVE NODULAR MASTOCYTOSIS WITH SYSTEMIC INVOLVEMENT. Lucio Bakos, Ronildo Storck and Ernesto Silveira Netto 635
AN UNUSUAL PRESENTATION OF DISCOID LUPUS ERYTHEMATOSUS. T. S. Haroon and K. A. Fleming 642

Quarterly Review

DRUG REACTIONS XX. CUTANEOUS REACTIONS TO ANTICONVULSANTS. John Almeyda and Ashley Levantine 646

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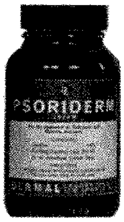

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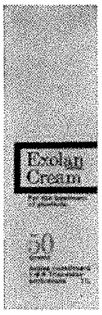

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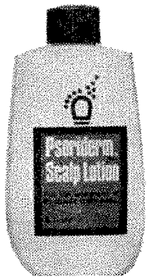

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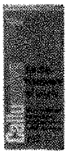

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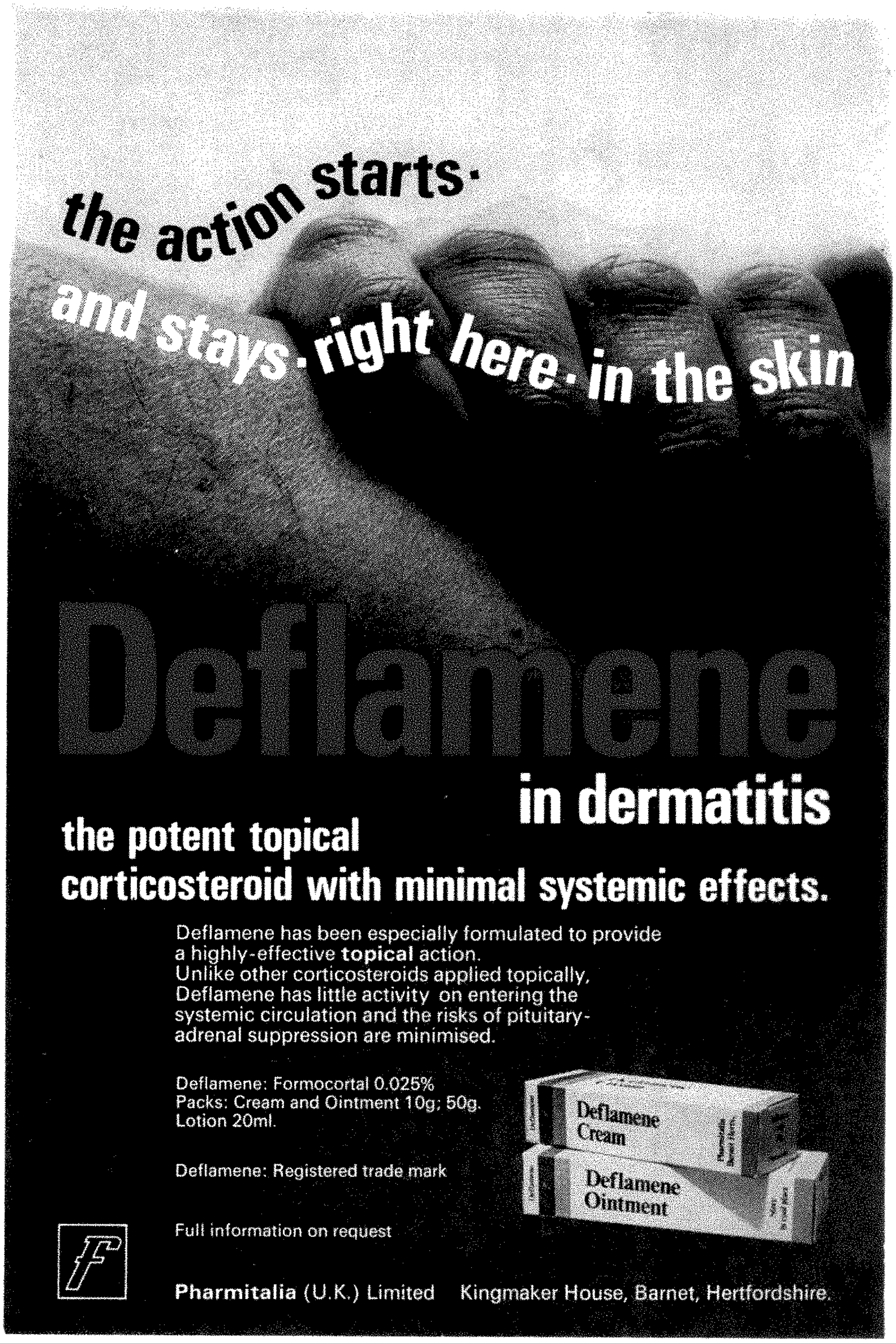

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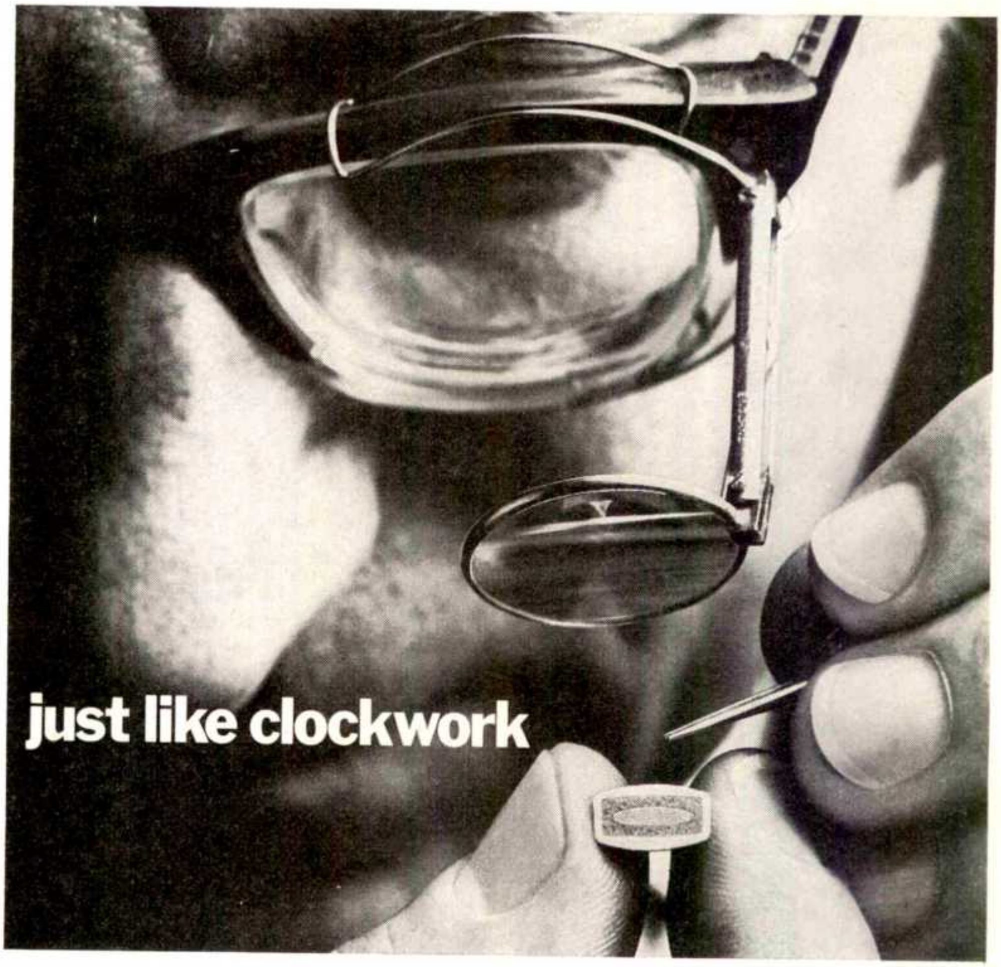

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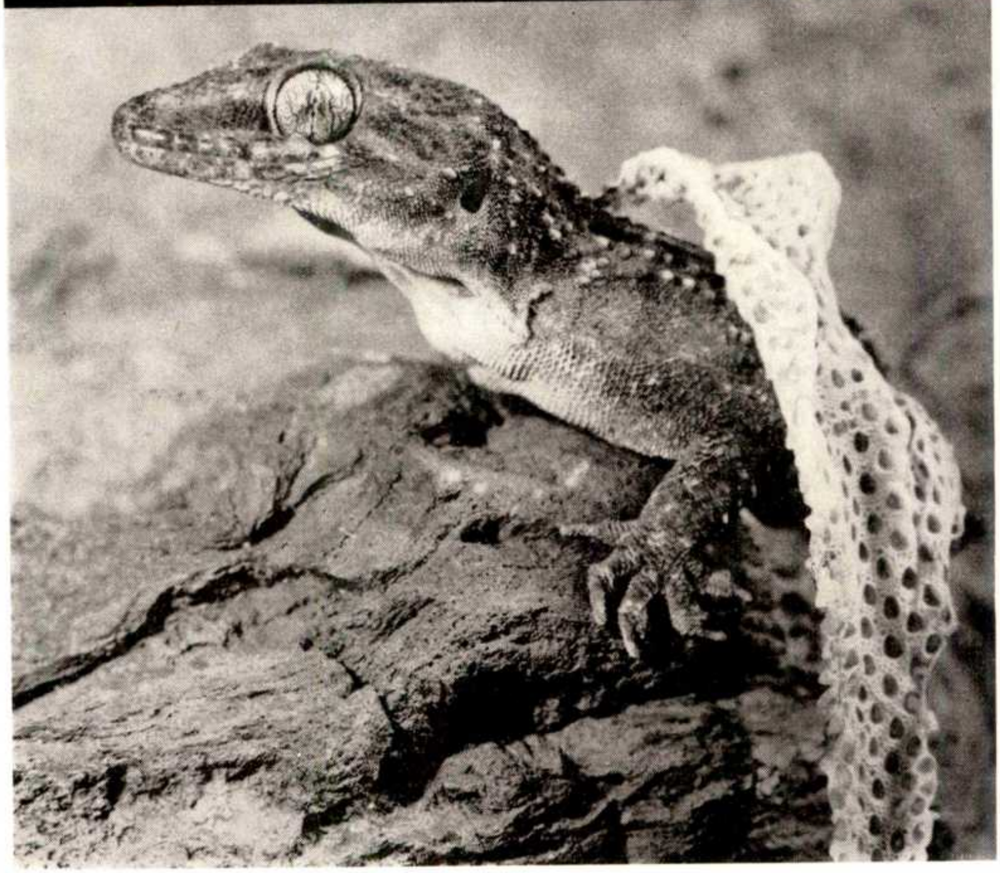

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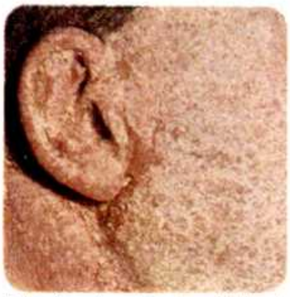
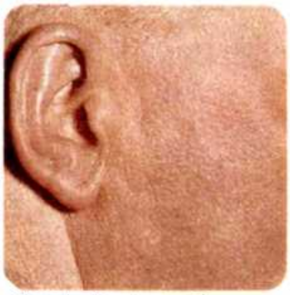
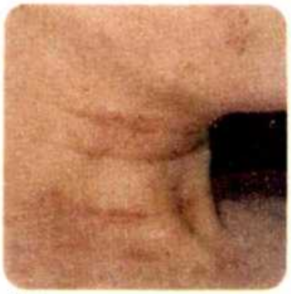
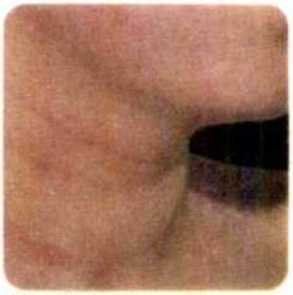

Seborrhoeic dermatitis since childhood


After 7 days' treatment with Betnovate Ointment


Atopic eczema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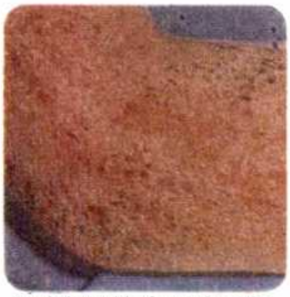
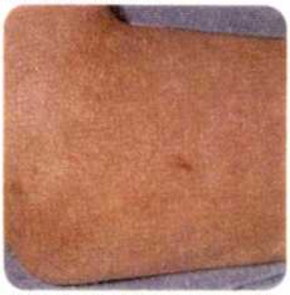
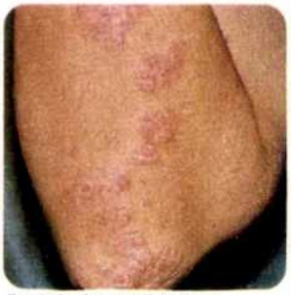
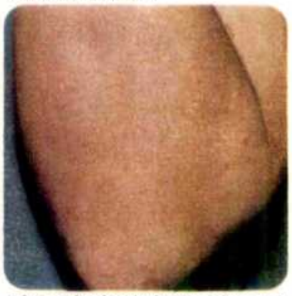
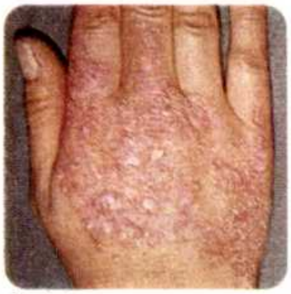
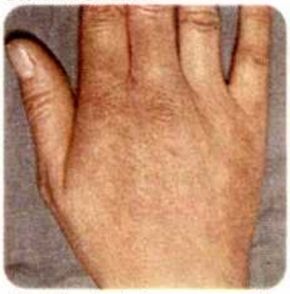

Atopic dermatitis of 3 years' duration

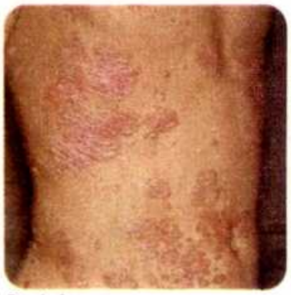

After 5 days' occlusive treatment with Betnovate Ointment

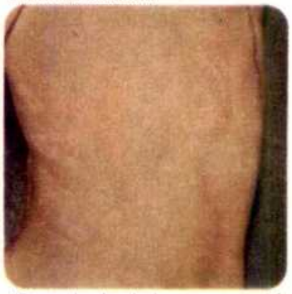

Psoriasis of 20 years' duration

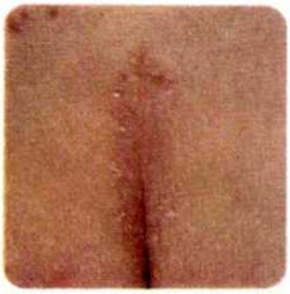

After 12 days' occlusive treatment with Betnovate Ointment

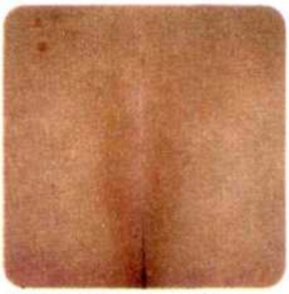

Psoriasis

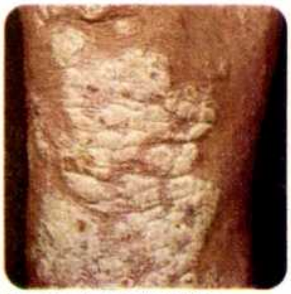

After 9 days' occlusive treatment with Betnovate Ointment

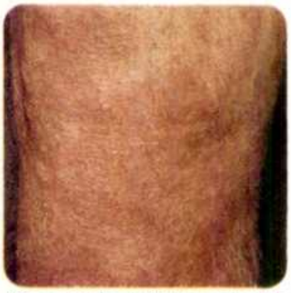

Psoriasis


After 21 days' occlusive treatment with Betnovate


Flexural psoriasis of 15 years' duration


After 4 weeks' treatment with Betnovate Ointment


Psoriasis of 51 years' duration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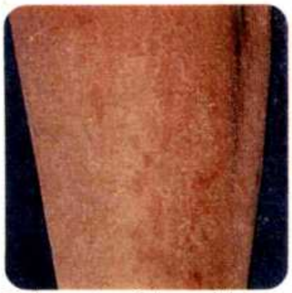
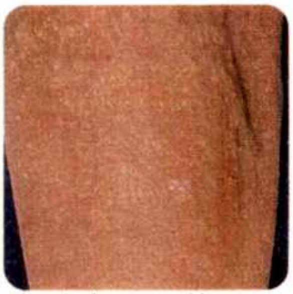
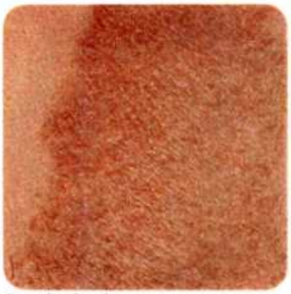


Varicose eczema of 4/5 months' duration

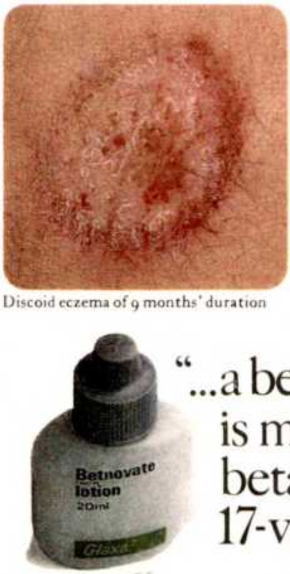


After 7 days' treatment with Betnovate Cream

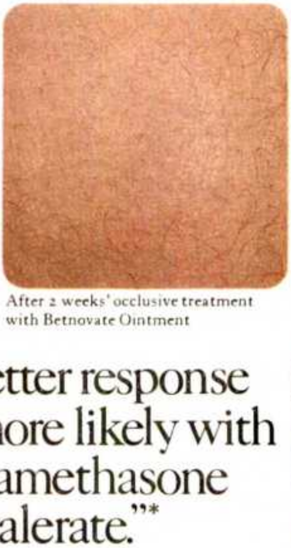


Constitutional eczema

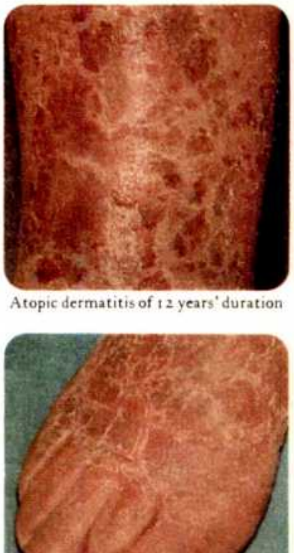
After 4 weeks' treatment with Betnovate Cream



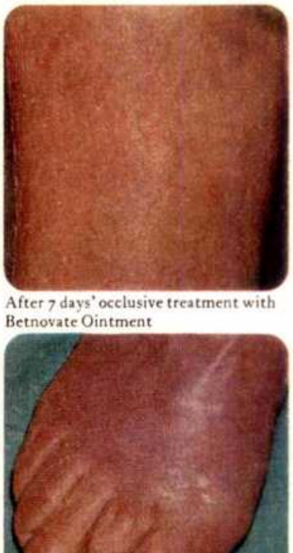
Discoid eczema of 9 months' duration



After 2 weeks' occlusive treatment with Betnovate Ointment



Atopic dermatitis of 12 years' duration



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*Lancet (1964) 1, 1,177

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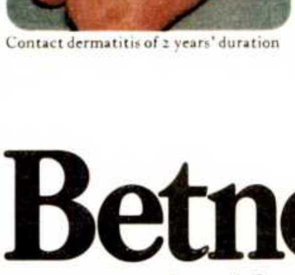
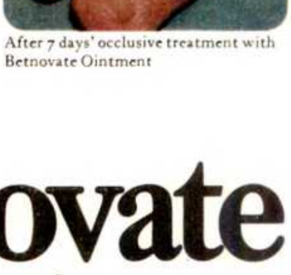


Contact dermatitis of 2 years' duration



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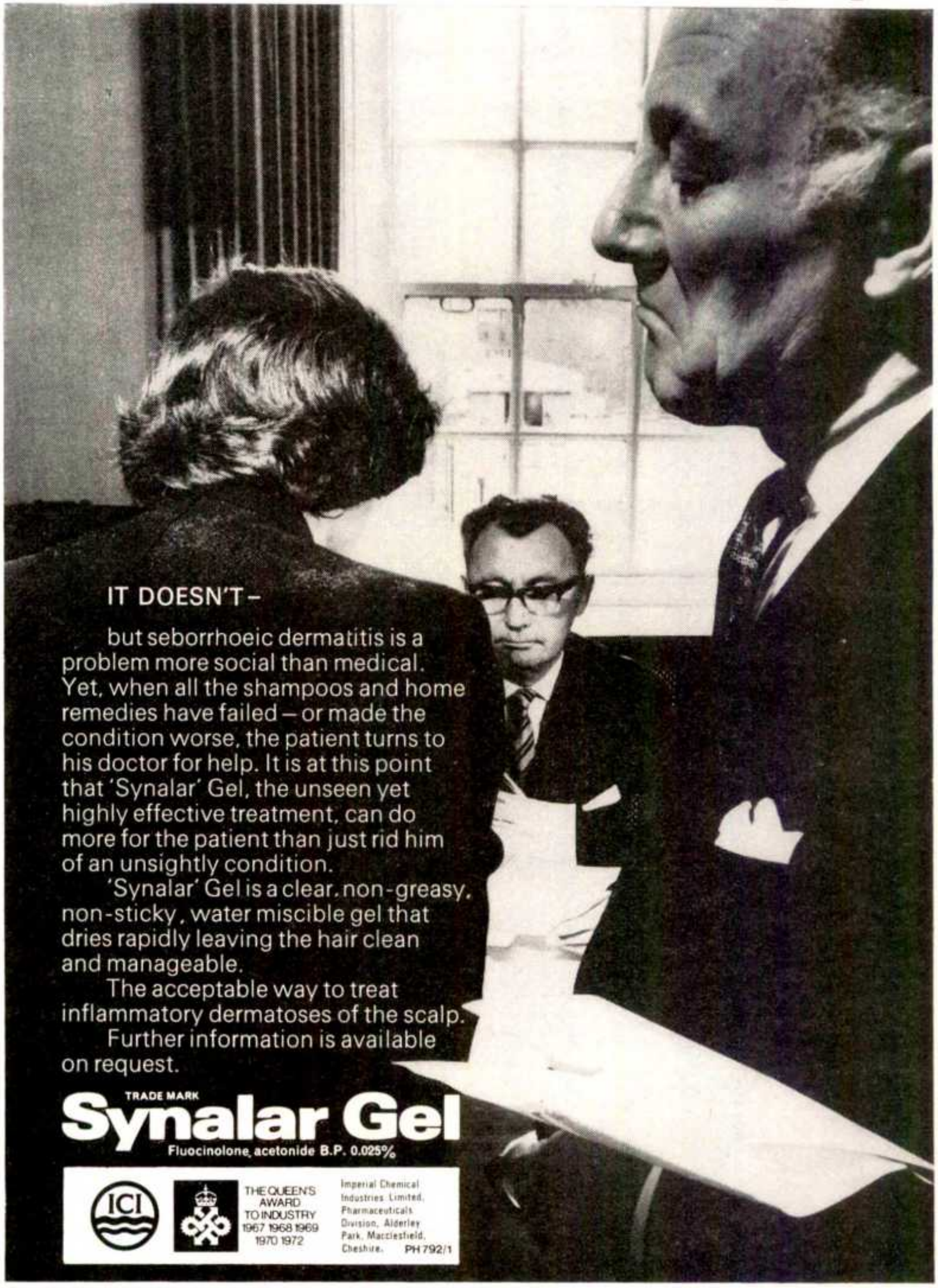

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Clinical and Laboratory Investigations

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University Department of Dermatology, Anderson College, and Western Infirmary, Glasgow

SENEAR-USHER SYNDROME AND INTERNAL MALIGNANCY

N. K. SAIKIA AND L. E. S. MACCONNELL

Summary.—Three cases of Senear-Usher syndrome are reported. All 3 had immunological evidence of co-existing pemphigus and lupus erythematosus. Two patients died of bronchial carcinoma. One surviving patient has probable metastatic neoplasia in the liver.

*

SINCE Senear and Usher (1926) first described this syndrome much has been written regarding its clinical interrelationship with pemphigus vulgaris and lupus erythematosus with bullae. The problem was reviewed under the title of " Pemphigus Erythematosus " by Senear and Kingery (1949), who concluded that the syndrome was a form of pemphigus. Lever (1965) supported this with his observation that pemphigus erythematosus could be histologically differentiated from acute or chronic lupus erythematosus (LE) with bullae.

The subject was reopened by Chorzelski *et al.* (1968) who found immunofluorescent (IF) characteristics of both systemic LE and pemphigus on skin specimens from 5 of 6 patients with Senear-Usher syndrome. Bean and Lynch (1970) reported similar findings in a single case.

To our knowledge there have been no previous reports of an association of the Senear-Usher syndrome with internal malignancy.

CASE REPORTS

Case 1. J.J. aged 67, presented in June 1968 with a 2 month history of circular skin lesions which started around the umbilicus and then spread to the trunk, limbs and scalp. There were scattered, slightly scaly, patches on chest, back and scalp and fresh erythematous lesions of the face. There was early finger clubbing, a history of slight weight loss and of a cigarette consumption of 40-50/day.

All investigations at that time, including a chest x-ray, were normal, apart from the histology of a forearm skin biopsy which showed an acute dermatitis reaction in the epidermis. Nevertheless a clinical diagnosis of Senear-Usher syndrome was made, and the skin responded initially to a local fluorinated steroid preparation.

He was followed up at Dunoon as an outpatient and readmitted to the Western Infirmary in September 1969 with a 19-month history of a generalised, itchy, blistering eruption and a 6-month history of indigestion, productive cough, occasional mild chest pain and undue breathlessness on exertion. On examination there were erythematous scaly lesions of the limbs, back and neck; Nikolsky's sign was positive. During his admission he produced frothy blood-stained sputum and developed congestive cardiac failure.

Investigations were as follows: F.B.C., W.B.C., serum urea, electrolytes, liver function tests (L.F.T.s), proteins and electrophoresis were normal; L.E. cell phenomenon was not

Accepted for publication February 9th, 1972.

demonstrated; E.S.R. was 33 mm/hour; cytology of the sputum revealed clumps of tumour cells. Tomography of the chest showed occlusion of the left upper lobe bronchus almost certainly due to malignant neoplasm. E.C.G. showed evidence of posterior myocardial ischaemia; histology of the chest skin showed the features of pemphigus foliaceus.

The patient improved on cobalt beam therapy to the tumour, diuretics and local corticosteroid preparations to the skin. He was discharged home in October, but pursued a steady downhill course until his death on December 25th, 1969.

Case 2. M.G., aged 48, presented at the Dermatology Outpatient Department in May 1970 with a 6-month history of a red, itchy eruption which began as blisters on the thighs and then spread to the trunk and limbs. Clinically, there were scattered, scaly, erythematous lesions on the trunk and limbs, more marked in the flexures. Nikolsky's sign was negative, and a tentative diagnosis of seborrhoeic dermatitis was made. Within a few weeks he developed lesions of the face and scalp, and histology of the anterior chest skin confirmed the clinical diagnosis of pemphigus foliaceus. The patient was generally ill, being extremely dyspnoeic, and had a complicated past history of chest trouble which began in April 1969 following a mass-radiography film which showed a pleural effusion. Virological studies showed complement fixation tests positive to mumps "S" and respiratory syncytial viruses. A pleural aspirate was reported as being positive for adenocarcinoma cells. He was admitted to the City Hospital, Edinburgh, for further investigation in May 1969, but the diagnosis of neoplasm was not substantiated, in spite of exhaustive investigations, including bronchoscopy, thoracoscopy and mediastinoscopy, all routine laboratory results being negative or normal, with the

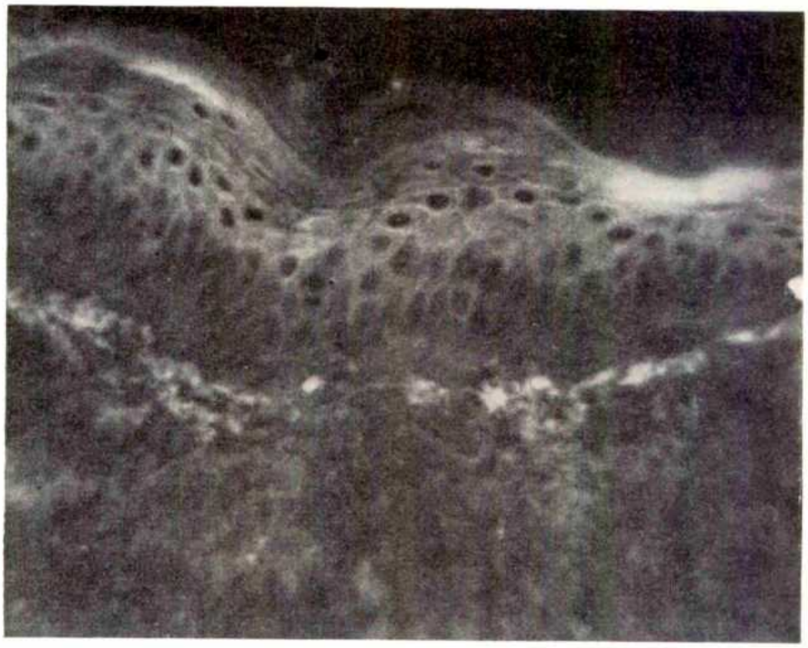

FIG. 1.—Direct I.F. test on patient's uninvolved skin from forearm (Case 3). Note intercellular immunofluorescence in the epidermis, characteristic of pemphigus and dermo-epidermal junctional I.F. as seen in L.E. The former is seen more prominently in the upper few layers of prickle cells (× 160).

exception of a strongly positive Mantoux test and radiological evidence of a right pleural effusion.

He was admitted to the Western Infirmary, Glasgow, in July 1970, where he was noted to have supraclavicular and axillary lymphadenopathy. A cervical node and an axillary gland were removed. Both of these were infiltrated by a moderately well differentiated adenocarcinoma. The pathology report stated " the appearances are consistent with the clinical diagnosis of carcinoma of the bronchus ". In view of the probability of the growth being disseminated, he was treated with a course of cyclophosphamide and prednisolone 20 mg daily. He was readmitted in October 1970 and again in December for radiotherapy. His last admission as an emergency on January 5th followed a terminal course until his death on January 13th, 1971.

Throughout his various hospital admissions, F.B.C., W.B.C., serum urea, electrolytes, L.F.T.s and proteins, thyroid scan, barium meal and intravenous pyelogram were within normal limits. E.S.R. was 60 mm/hour; L.E. cell phenomenon was not demonstrated. Chest x-rays were relatively little changed throughout his last year, showing bilateral consolidation and the presence of an increasing amount of fluid in the pleura.

Case 3. D.McD., aged 70, presented in June 1971 with a 4-month history of itchy blisters on the scalp, arms and legs. Apart from a small ulcerated lesion on the lower lip, treated locally 18 years previously, and long-standing nocturia, he was asymptomatic. On examination on admission, he was an elderly, weather-beaten man, with marked arcus senilis, bilateral deafness and a widespread, crusting, erythematous eruption which spared the forearms, hands, calves and feet. Nikolsky's sign was positive.

Histology of the lip lesion was of an early invasive squamous carcinoma arising on an actinic carcinoma *in situ*. This was satisfactorily treated with radium needles. Histological examination of a skin biopsy from the left scapular region confirmed the clinical diagnosis of pemphigus foliaceus. The patient responded well to a local dilute fluorinated steroid preparation and systemic prednisolone 20 mg daily, reducing to a maintenance dose of 5 mg daily within 3 weeks.

Other investigations were as follows: E.S.R., 83 mm/hour; S.G.O.T. and S.G.P.T. were 96 and 71 Frankel units per ml respectively; serum albumin and globulins were 3·2 and 3·0 g per 100 ml; electrophoresis showed increased α-$_2$ and γ-globulins; urea was 58 mg per 100 ml; bromsulphthalein was equivocal, 6% dye being present at 45 min; F.B.C., W.B.C., platelets, serum electrolytes, bilirubin, alkaline and acid phosphatases, skull x-ray and barium enema were all normal. L.E. cell and latex phenomena were not demonstrated. Chest x-ray showed severe emphysema, but no active pathology. Barium meal demonstrated marked deformity of the duodenal cap consistent with chronic ulceration, although no active ulcer crater was seen. On scintiscan, there was marked irregular reduction of uptake throughout the liver in keeping with widespread neoplastic involvement or advanced cirrhosis; but, since there was absence of splenic uptake, the former aetiology was considered more likely. Ultrasonography showed a slightly bulky liver, with minimal increase in the diffuse echo pattern and a spleen size at the upper limit of normal. There was no evidence of ascites.

IMMUNOLOGICAL INVESTIGATIONS

Materials

(1) Sheep anti-human IgG conjugated with fluorescein isothiocyanate (F.I.T.C.).

(2) Human vaginal epithelium and guinea-pig's lip were used as the substrates.

Methods and Results

(1) *Direct I.F. tests* on patient's skin were performed according to the method described by Cormane *et al.* (1964, 1970) and Burnham *et al.* (1963). Characteristic inter-

cellular immunofluorescence of pemphigus and dermo-epidermal junctional immunofluorescence as seen in L.E. were present in all 3 cases.

(2) *Indirect I.F. tests.* (i) Antinuclear factor (Frew *et al.*, 1958; Holborow *et al.*, 1957) was weakly positive at 1 : 16 titre in all cases. (ii) Intercellular immunofluorescence (Beutner *et al.*, 1968) was positive in all 3 cases with titres as shown in Table I.

TABLE I

Case no.	Case	Titre dilution
1	J.J.	1 : 80
2	M.G.	1 : 60
3	D.McD	1 : 128

DISCUSSION

The IF studies on these patients confirm the findings described by Chorzelski *et al.* (1968) and Bean and Lynch (1970), who suggested that the Senear–Usher syndrome is a combination of LE and pemphigus foliaceus.

An association of malignant disease with the Senear–Usher syndrome has not yet been described in the literature. The 3 cases presented here are the only ones of Senear–Usher syndrome seen in our department in the past 4 years. Whether or not the proven malignancy in 2 of them is a significant finding when comparing the general population of a similar age group in the West of Scotland is difficult to ascertain, as these patients are in different age groups. It would seem to be more significant if the third case eventually proved to have a primary tumour giving rise to secondary deposits to account for the liver findings.

We think it probable that there is the production of pemphigus antibodies by a tumour in these cases, as one of us recently reported a case of pemphigus vulgaris with malignant lymphoid neoplasm in which a possible interrelation of the tumour with pemphigus was established *post mortem* (Saikia, 1972). Unfortunately, we could not obtain permission for post-mortem immunological investigations in the 2 patients reported here who died. The third patient, who has abnormal liver function, high E.S.R. and a liver uptake suggestive of metastatic deposits, admits to a high alcohol intake. A diagnosis of cirrhosis of the liver cannot, therefore, be excluded. Liver biopsy was not done in this patient due to his age, emphysema and socio-economic obligations requiring his early discharge. He is, however, being kept under observation as an outpatient.

We are indebted to Dr M. A. Ratzer and Dr R. L. Cormie for permission to quote the clinical details of their patients, and to Dr J. Kerr for permission to quote the chest findings in Case 2.

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ALLERGIC CONTACT DERMATITIS TO GARLIC

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Summary.—A case of contact dermatitis to garlic is described. Patch tests with a piece of garlic bulb and extracts of garlic were positive. A tulip extract gave a positive reaction in this patient, but extracts of onion did not do so. The active substance proved to be readily soluble in water, alcohol and acetone. The allergenic material was found to be of low molecular weight, heat labile and present in the outer parts of the bulb.

VEGETABLES and fruits may lead to several types of allergic phenomena. Their *consumption* may provoke immediate adverse reactions, especially in atopic individuals (Bleumink, 1970). In such cases the eliciting agents are of (glyco) protein nature. In addition the *handling* of some vegetables and fruits may lead to allergic contact dermatitis. In this type of allergy the responsible agents are generally of low molecular weight and soluble in acetone, ethanol or ether.

The essential oils of the edible *Umbelliferae*: carrots (*Daucus carota* L.), parsnips (*Pastinaca sativa* L.), parsley (*Petroselinum crispum* Nym.), and celery (*Apium graveolens* L.) may cause allergic contact dermatitis in sensitized individuals (Klauder and Kimmich, 1956; Fisher, 1967). Terpenes have been incriminated as sensitizing agents in dermatitis caused by oranges and lemons (Fisher, 1967). Less known is allergic contact dermatitis to onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Both vegetables are members of the *Liliaceae* family.

The first description of garlic dermatitis dates from 1950. It is a case report by Edelstein (1950) of dermatitis on the wrist of a meatgrinder. Burgess (1952) described a case of contact dermatitis of the fingertips and thumb of a cook aged 30. Patch tests with pieces of onion and garlic were positive. Burks (1954) performed patch tests in 10 persons with probable onion or garlic dermatitis. A water extract of garlic gave positive skin reactions in 8 patients; 4 reacted positively to an onion extract; no positive reactions were observed in 10 control persons.

We wish to report a case of severe contact dermatitis caused by garlic. Attempts were made to characterize the active substance.

CASE REPORT

A man aged 30, who owned several restaurants, complained for about 7 months of eczema on both hands. The patient reported that the process started between the third and fourth finger of the left hand. In spite of treatment with external corticosteroid therapy by several dermatologists, the process spread over both hands. At his first visit to our clinic both his hands were oedematous, red, swollen and itchy. The nails showed several transverse depressions (Beau's lines).

Further questioning revealed that he worked occasionally as a cook in his own restaurants, making new sauces. Moreover, the start of his eczema coincided with the

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production of a new relish called " Spanish Sauce ". Therefore, patch tests were done with the ingredients of this sauce: paprika, " saté " spices, garlic, curry, " aroma salt ", grill spices, " saté mix " and soup concentrates. The substances were tested as such. Patch tests were performed according to the methods adopted by the International Contact Dermatitis Research Committee (Malten, 1971).

Positive reactions were observed exclusively with garlic. A patch test with a small piece of onion was also found to be positive; a tulip bulb gave only a weakly positive reaction. Routine patch testing, including the allergens 0001–0020 recommended by the above mentioned Committee (Malten, 1971) were all negative.

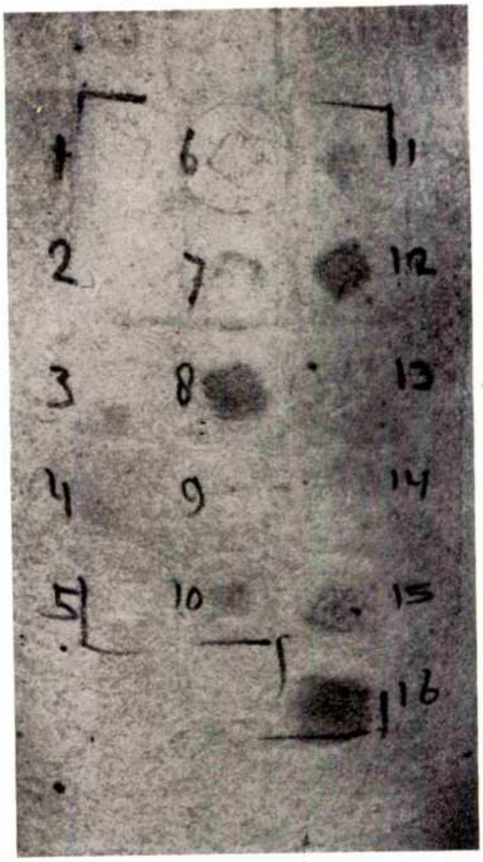

FIG. 1.—Patch tests with garlic, onion and tulip extracts.

1–7	Control solutions
8	Aqueous garlic extract
9	*Ibidem* heated at 100°
10	*Ibidem* dialysed
11	*Ibidem* treated with acid
12	Acetone extract of intact garlic bulbs
13	Onion, water extract
14	Onion, ethanol extract
15	Acetone extract of intact tulip bulbs
16	Garlic, ethanol extract.

Nature of the Contact Allergen

In addition to the tests with small pieces of garlic, onion and tulip, skin tests were performed with extracts obtained from these materials.

The following extracts were prepared:

(1) Garlic (21 g) was pulverized in a garlic press and extracted by vigorous shaking during 2 hours with 50 ml distilled water, after which the suspension was centrifuged. This procedure was repeated twice. The clear supernatants were collected and lyophilized. Yield: 5·7 g white-yellow powder.

(2) Extraction with 3 × 50 ml 96% ethanol of a similar amount of garlic. The resulting solution was concentrated 10 times in a vacuum rotator evaporator.

(3) Extraction with 3 × 50 ml acetone, as (2) above.

(4), (5) and (6) Extraction of 18 g garlic powder (commercially available product which is prepared by drying the press fluid of garlic) with water, ethanol and acetone respectively. Compare (1), (2) and (3). Yield of 4 : 8·2 g lyophilized material.

(7) Onion (40 g) was cut in small pieces and macerated 3 times with 100 ml water. Yield: 3·2 g hygroscopic powder.

(8) Onion (40 g) extracted with 3 × 100 ml 96% ethanol. The solution obtained was evaporated to about 30 ml.

(9) Tulip bulbs from which the brown peel had been removed were treated with 80% acetone after the method described by Verspyck Mijnssen (1968). To this end 185 g of intact bulbs (Darwin tulips, *var.* Rose Copland) were shaken during 2 hours with 2 × 375 ml 80% acetone. The resulting extract was concentrated under vacuum in a rotator evaporator to about 10 ml.

(10) Intact garlic bulbs (200 g) were extracted with 2 × 400 ml acetone, as described under (9) above. About 1·2 g brown hygroscopic powder was obtained.

(11) Finally an aqueous garlic extract (Extract (1) above) was subjected to several chemical treatments. 1 g of Extract (1) was dissolved in 10 ml distilled water and dialysed during 48 hours in a cold room (4°C) against several changes of distilled water. The resulting non-dialysable material was lyophilized (yield 660 mg).

(12) Five hundred mg of garlic, Extract (1), was dissolved in 10 ml water and the solution heated during 15 min in a boiling waterbath. The precipitate was removed by centrifugation and the supernatant lyophilized.

(13) Five hundred mg of garlic extract was dissolved in 0·2 N H_2SO_4 and heated during 4 hours at 50°C. The resulting solution was neutralized with NaOH and lyophilized.

The results of the patch tests with these substances are summarized in Table I.

TABLE I.—*Patch Tests with Garlic, Onion and Tulip Extracts**

Code	Source of the allergen solution	Nature of the extract	Test solution	Patch test results (48 hours)	Results (72 hours)
1.	Garlic pulverized	Water	400 mg/ml	3+	2+
2.	*Ibidem*	Ethanol	Conc. 10 ×	3+	2+
3.	*Ibidem*	Acetone	Conc. 10 ×	3+	2+
4.	Garlic powder	Water	500 mg/ml	3+	2+
5.	*Ibidem*	Ethanol	Conc. 10 ×	—	—
6.	*Ibidem*	Acetone	Conc. 10 ×	—	—
7.	Onion, pulverized	Water	100 mg/ml	—	—
8.	*Ibidem*	Ethanol	Conc. 10 ×	—	—
9.	Tulip bulbs	80% acetone	Conc. 75 ×	+	+
10.	Garlic bulbs	80% acetone	Conc. 75 ×	2+	2+
11.	Aqueous garlic extract, dialysed		400 mg/ml	±	—
12.	Aqueous garlic extract, heated		400 mg/ml		—
13.	Aqueous garlic extract, treated with H_2SO_4		400 mg/ml	±	—

* The extracts were also tested in 8 control individuals. In one of these Extract 1 showed a weakly (±) positive reaction; all other extracts were negative.

DISCUSSION

The patch tests show that the active sensitizer is readily soluble in water, ethanol and acetone. Burks (1954) found the allergen in garlic to be water-soluble, but no activity was demonstrable in an ether extract. Of the extracts obtained from garlic powder (dried press fluid from garlic) only the aqueous one showed skin reactivity. It is apparent, therefore, that the allergen is hydrophilic. Moreover, the skin tests indicate the active component to be of low molecular weight and heat labile. It is inactivated by heating at 100°C during 15 min. Domestic cooking will therefore destroy the active substance. Treatment with dilute acid also leads to inactivation of the allergenic material.

Our results indicate that the potent sensitizer of garlic is present in greatest concentration in the outer parts of the bulb. Identical results were obtained by Verspyck Mijnssen (1968), who showed that large quantities of the allergenic material, causing " tulip fingers " in individuals allergic to tulip, are present directly underneath the brown peel of the bulbs.

Garlic is a member of the family of the *Liliaceae*, to which onion and tulips also belong. It is therefore tempting to assume that garlic, onion and tulip (*Tulipa gesneriana* L.) contain similar or cross-reacting allergens.

In the study of Burks (1954) only 4 of the 8 patients with strong reactions to garlic had weakly positive reactions to onion. Similarly, extracts from onion, prepared in the same way as the water and ethanol test solution from garlic, did not show activity in our patient. In any case, if similar allergens are present in both vegetables, the amount of active material present in onion must be much lower. In our patient a tulip extract was also found capable of eliciting positive skin responses. Verspyck Mijnssen (1968) presented evidence that the active allergenic component in tulips bulbs is the glycoside of α-methylene-β-butyrolactone. Because this substance was not available, we were not able to test our patient with it. Besides further chemical analyses of the active garlic allergen we are planning now to perform patch tests in groups of patients with tulip fingers and garlic dermatitis with both (purified) tulip and garlic allergens. The results of these studies will be published in due course.

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THE EFFECT OF ENZYME DETERGENTS ON TRANSEPIDERMAL WATER LOSS FROM NORMAL SKIN

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Summary.—Transepidermal water loss from normal forearm skin was measured in 15 normal subjects before and after a standardized " skin insult " produced by friction with a detergent alone or with the detergent plus proteolytic enzyme. No statistically significant difference was discernible between the results obtained with the two solutions.

A METHOD of producing a standard amount of primary irritant skin damage using a detergent in a modification of Vermeer's washing simulator has been previously described (Raffle and Shahidullah, 1970), and it was shown that a slight but significant increase in transepidermal water loss (TWL) from normal forearm skin could be obtained as a result. There has been interest recently regarding the potentially damaging effect on the skin of detergents containing proteolytic enzymes. It was decided to compare the effect of detergent alone and detergent plus enzyme on TWL from normal skin to obtain objective evidence as to whether enzyme detergents damaged the barrier function.

METHOD

The standard " skin insult " with detergent and detergent plus enzyme was produced by a modified Vermeer washing simulator. Two 1% w/v (pH 10·2) solutions were made up from detergent powder with enzyme and detergent powder without enzyme. These were complete fabric washing detergent products, and the formulation of both was similar except that one contained enzyme. They were rubbed on to the skin of the volar aspect of the lower third of the forearm at 37°C in the washing simulator for 10 min. Identical skin sites were used, the detergent solution being applied to the left forearm and the detergent with enzyme solution to the right forearm. The solutions were freshly made, since the half-life of the enzyme is 2 hours. TWL from the selected sites was measured prior to the application of solution, 4–6 hours after the application, and again 24 hours after the application. Ambient temperatures at the time of recording ranged from 14–20° C and humidities from 65–80%. The electrolytic hygrometric method employed for monitoring TWL has been described previously (Shahidullah *et al.*, 1969). Sweat suppression was carried out by the application of 5% poldine sulphate in alcohol 6–12 hours before the test, and again one hour before the test. By this means sweat suppression is achieved for at least 24 hours. Tests were carried out on 15 subjects, 11 female and 4 male, all of whom were free from skin disease.

RESULTS

Examination of the skin sites after the period of " washing " revealed only very minor changes. In 2 subjects there were minimal transient erythema and oedema with both solutions, but the remainder showed no detectable morphological

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changes. The TWL values before and after the "skin insults" are shown in Table I. With the detergent solution alone, 4–6 hours after the insult, slight rises in TWL (0·02–0·22 mg/cm^{-2}/hour) were noted in 8 subjects, no change in 3, and slight falls (0·02–0·14 mg/cm^{-2}/hour) in 4. These changes in TWL were not sustained after 24 hours. With the detergent plus enzyme, slight rises in TWL (0·02–0·34 mg/cm^{-2}/hour) were noted in 10, and slight falls (0·02–0·12 mg/cm^{-2}/hour) in 5 subjects. Again these values were not sustained after 24 hours. Statistical analysis of these figures shows no significant increase in TWL values, either with detergent alone or detergent plus enzyme, and no significant difference between the detergent plus enzyme and the detergent alone groups after 4–6 hours or after 24 hours.

TABLE I.—*Transepidermal Water Loss* (mg/cm^{-2}/hour)

Subject	Sex	Site*	Initial recording	4–6 hours after skin insult	24 hours after skin insult
1	F	L	0·48	0·50	0·46
		R	0·42	0·56	0·38
2	F	L	0·36	0·34	0·28
		R	0·40	0·28	0·40
3	F	L	0·50	0·60	0·48
		R	0·48	0·38	0·30
4	F	L	0·70	0·60	0·60
		R	0·74	0·76	0·58
5	F	L	0·34	0·36	0·32
		R	0·30	0·48	0·34
6	F	L	0·44	0·44	0·50
		R	0·42	0·54	0·50
7	F	L	0·34	0·32	0·34
		R	0·30	0·40	0·36
8	F	L	0·34	0·34	0·36
		R	0·32	0·48	0·40
9	F	L	0·40	0·48	0·42
		R	0·38	0·48	0·42
10	F	L	0·36	0·50	0·46
		R	0·36	0·50	0·42
11	F	L	0·52	0·60	0·50
		R	0·50	0·44	0·48
12	F	L	0·52	0·38	0·40
		R	0·48	0·44	0·44
13	M	L	0·36	0·38	0·36
		R	0·46	0·50	5·58
14	M	L	0·70	0·92	0·76
		R	0·76	1·10	0·80
15	M	L	0·38	0·38	0·38
		R	0·38	0·36	0·38

* L = from left forearm site (detergent alone); R = from right forearm site (detergent plus enzyme).

DISCUSSION

Previously a 6% solution of triethanolamine lauryl sulphate was shown to produce under similar circumstances a slight but statistically significant increase in TWL in normal forearm skin in 22 subjects (Raffle and Shahidullah, 1970). However, neither the detergent nor detergent plus enzyme produced statistically significant increases in TWL values either 4–6 hours or 24 hours after the insult.

Moreover, little or no observable change was seen in the skin as the result of the skin insults with detergent or detergent plus enzyme. It may well be that concentrations greater than 1% w/v could be shown to produce significant increases in TWL, but since this concentration represents twice the concentration advised by the manufacturers for household use, the evaluation of higher concentrations was not pursued.

These findings indicate that in normal skin the barrier function, as evidenced by TWL, is not disturbed by concentrations of detergents and proteolytic enzymes in excess of that advocated for use by housewives. Griffith *et al.* (1969) were unable to demonstrate untoward effects from enzyme-detergent mixtures in large numbers of subjects. Bolam *et al.* (1971), in a clinical assessment of enzyme detergents in over 4000 housewives, showed that detergents containing proteolytic enzymes had no greater effect on the skin than conventional detergents, and no adverse reactions attributable specifically to the enzyme products were seen. However, cases of dermatitis attributed to enzyme washing powders have been reported (Jensen, 1970; Cochran, 1970; Ducksbury and Dave, 1970; Bamji and Bamji, 1970; Wuthrich *et al.*, 1971). The findings reported here do not exclude the possibility of unexpected reactions to proteolytic enzymes occurring in certain individuals, and it may be that a newly formed barrier layer following dermatitis may be more susceptible to such substances than that of normal skin.

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HUMAN EPIDERMAL CELL CYCLE *IN VITRO*

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Summary.—Human epidermal cells were propagated *in vitro*, pulse labelled with tritiated thymidine, fixed at regular intervals thereafter and processed for radioautography. The percentage of labelled mitoses was plotted against time after pulse. From the interval between 2 successive peaks on the curve of labelled mitoses, the length of the cell cycle was found to be 59 hours. The growth fraction or percentage of cells actually involved in proliferation was calculated to be approximately 53·9%. The data are discussed with respect to published data on the human epidermal cell cycle *in vivo* and the methods by which the data were obtained.

CELL cycle kinetics of normal human epidermis have not been adequately investigated. The dearth of information *in vivo* is due, in part, to the low level of mitotic activity in the basal germinal layer, which has made it difficult to obtain reliable quantitative data. Investigations are also limited by the amount of radioisotope that can be injected into normal human subjects, the route of isotope administration, and the number of skin biopsies that can be obtained, thus precluding the type of comprehensive experiment possible in laboratory animals. Since there are many diseases of the human epidermis characterized by abnormalities of cell proliferation and differentiation, it would be useful to have a model system *in vitro* where the relation between these parameters could be investigated. Human epidermal cells have been propagated as outgrowth cultures *in vitro* and, under these conditions, seem to retain most of the fundamental attributes of the epithelium from which they were derived (Flaxman *et al.*, 1967; Friedman-Kien *et al.*, 1966). The culture grows as a stratified squamous epithelium in which only those cells in the bottom layer attached to the substratum (glass coverslip or plastic Petri dish) synthesize DNA and divide. A certain proportion of these dividing cells leave the germinal layer, move upward and contribute to the multilayered arrangement, and ultimately differentiate by keratinizing. Thus outgrowth cultures of human epidermis *in vitro* could serve as a useful system for studying cell proliferation and its relationship to cytoplasmic differentiation in tissues that show continual cell renewal. In the present work we have studied the cell cycle of human epidermis *in vitro* at an early stage of culture growth before the multi-layered, differentiating phase has been reached.

MATERIALS AND METHODS

Split thickness pieces of normal human abdominal skin, obtained during surgical procedures, were explanted on glass coverslips and cultured according to a method described previously (Flaxman *et al.*, 1967). After 4 days, an outgrowing sheet of

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epithelial cells appeared around the explants. The cells have previously been determined to be epidermal in origin. Experiments were performed on 7-day-old cultures showing minimal cell overlap, and thus minimal development of a stratified epithelium (Fig. 1).

For cell cycle studies tritiated thymidine (H^3TdR-sp. act. 6·7 Ci/mmol) was added to a final activity of 2 μCi/ml culture fluid. After a 2-hour pulse, cultures were removed from the radioactive medium, washed 3 times in " cold " culture fluid and incubated at 37°C in a fourth change of " cold " culture fluid. At appropriate intervals cultures were fixed in 10% buffered formalin. They were dehydrated, air dried, and the explants removed. For autoradiography, coverslips were mounted on microscope slides, dipped in Kodak NTB2 emulsion and exposed for 2 weeks at 4°C. Autoradiographs were stained in acid haematoxylin, and mitoses (Fig. 2) were counted with the aid of an oil immersion lens at a magnification of 1000 ×.

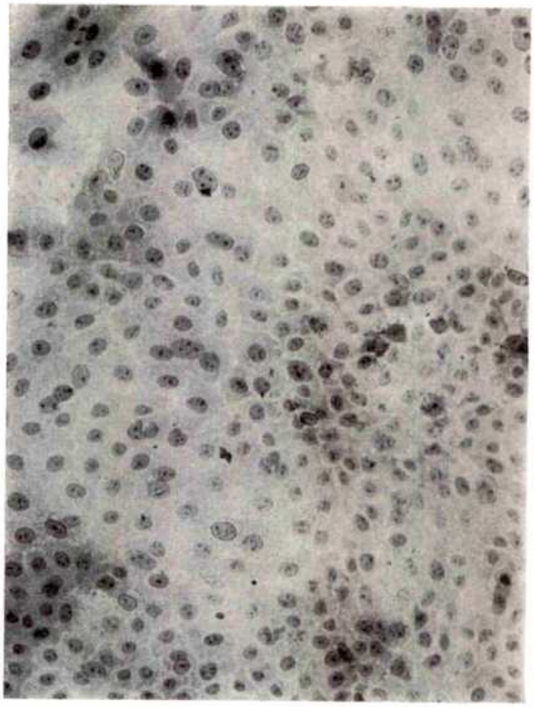

FIG. 1.—Light micrograph, showing portion of outgrowth of epidermal cells (× 120).

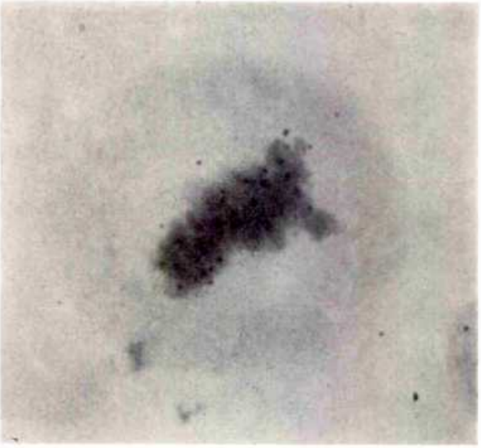

FIG. 2.—Labelled metaphase in outgrowth of epidermal cells (× 1200).

RESULTS

The experimental curve of percentage labelled mitoses versus time is shown in Fig. 3. Each point on the curve represents 5 cultures in which all mitoses (50–150) were counted. Total cell cycle time (T_c) was measured as the interval between the 2 successive peaks of labelled mitoses (at 13 and 72 hours respectively) and is about 59 hours. The duration of DNA synthesis (S) is 11·5 hours, as calculated from the interval between the 50% points on the ascending and descending limbs of the first curve. The post-synthetic phase (G_2) has a minimum value of 7 hours and represents the time between the beginning of the pulse and the appearance of the first labelled mitoses. Mitosis (M) lasts 1·5 hours, the difference between the pulse and 50% labelled mitoses minus G_2. G_1 is 39·5 hours, and is calculated as the difference between T_c and the sum of S, G_2 and M.

The labelling index ($L.I.$) was 105 ± 22 (10·5% ± 2·2%), and is expressed as the number of labelled nuclei per 1000 nuclei immediately after the pulse. The mitotic index ($M.I.$) was 25 ± 5/1000 cells, determined by counting metaphases arrested in the presence of colcemid (2 μg/ml) over a 4-hour period.

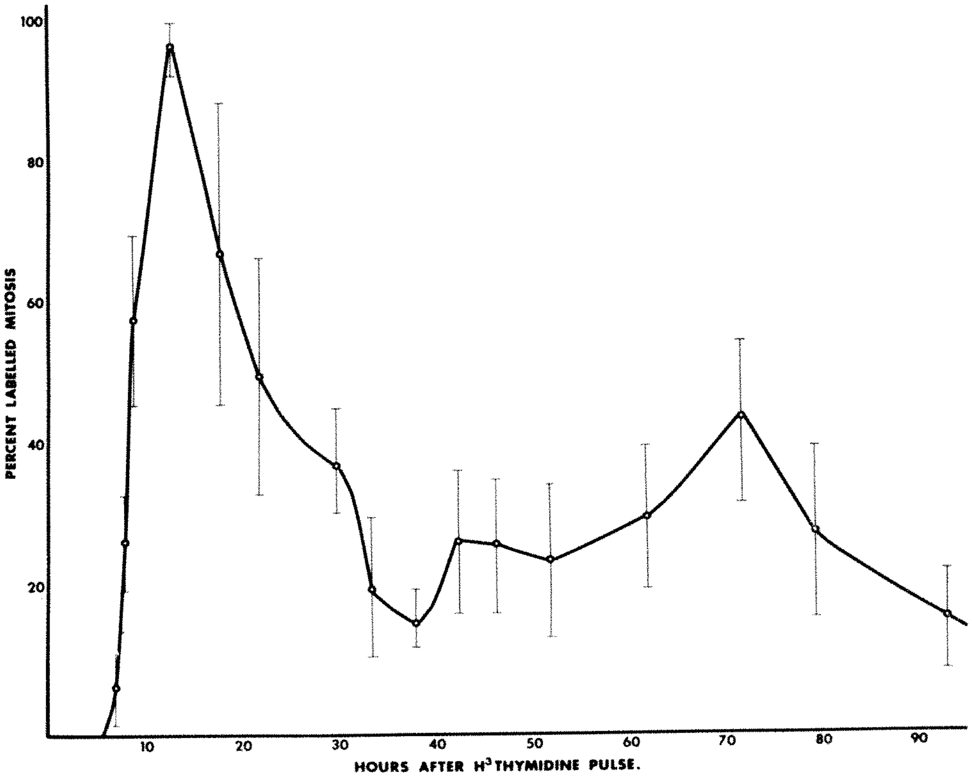

FIG. 3.—Percentage labelled mitoses and standard deviation at various intervals after pulse labelling with H^3 thymidine in outgrowth cultures of normal human epidermis *in vitro*.

Because T_c *in vitro* has been determined directly from the successive labelled mitotic peaks, the germinal pool or growth fraction ($G.F.$) can be calculated from the formula (Frindel *et al.*, 1968):

$$T_c = \frac{S \times G.F.}{L.I.} \tag{1}$$

$$59 = \frac{11{\cdot}5 \times G.F.}{10{\cdot}5}$$

$$G.F. = 53{\cdot}9\%$$

Therefore only approximately 54% of cells that appear germinal in 7-day-old cultures really are in the proliferative pool. This figure was checked by means of continuous labelling experiments where 7-day-old cultures were incubated with H^3TdR for 72 hours. Only 65% of the total population of cells became labelled. Since all cells in the proliferative pool should have divided once in this period of time, they would come to represent a higher proportion of the total number of cells at 10 days than at 7 days; thus the real proliferative pool at 7 days would be less than 65%.

DISCUSSION

Table I compares values from the present experiments *in vitro* with data obtained *in vivo* from normal human epidermis (Van Scott and Ekel, 1963; Weinstein and Frost, 1968, 1969). After 7 days *in vitro*, the epidermal cell cycle appears to be much shorter than *in vivo* because of a striking decrease in the length of G_1. At first glance this would seem to be reasonable since, *in vitro*, it is generally assumed that cells escape from the naturally present inhibitors of mitosis. As will be discussed, the data are not entirely comparable, and it may be questioned whether there is indeed a significant shortening of the cell cycle *in vitro*.

TABLE I.—*Comparison of Cell Cycle Kinetics of Normal Human Epidermis* in vivo *and* in vitro

	In vitro	*In vivo*
T_c	59 hours	308 hours
G_1	39·5 hours	284 hours
S	11·5 hours	16 hours
G_2	7 hours	6–8 hours
M	1·5 hours	1 hour
L.I.	105±22	52±15
M.I.	25±5	8·6
G.F.	54%	?

In the present study T_c was directly determined by measuring the time between 2 successive peaks of labelled mitoses, a method that is independent of the *G.F.* of the culture (Baserga and Malamud, 1969). On the other hand, the value obtained *in vivo* by other investigators, using only a single curve of labelled mitoses, was calculated from a formula (Weinstein and Frost, 1968, 1969):

$$\frac{N_s}{T_s} = \frac{N_c}{T_c} \qquad (2)$$

where N_s = number of labelled nuclei
T_s = duration of S
N_c = total number of germinative cells
T_c = duration of cell cycle.

Using this formula, *G.F.* must be known in order for T_c to be calculated with any degree of confidence. The T_c value of 308 hours *in vivo* is based on the assumption that N_c is equivalent to 100% of the cells counted in the epidermal basal layer. However, there is not yet any published data showing that this is indeed the case. If a significant percentage of epidermal basal cells *in vivo* were outside the cell cycle in a resting state, T_c, according to the formula, would be shorter than 308 hours, the actual value depending on the real size of the pool.

In the present experiments only 54% of the cells that appear " germinal " in 7-day-old cultures actually are in the proliferative pool as calculated from formula

(1). For the same reasons that the *G.F.* after 3 days of continuous exposure to H^3TdR is probably too high, the derived *G.F.* at 7 days probably represents an increase over that present at the moment of explantation. Further evidence in support of the existence of a non-dividing population comes from our observations that, within the 7-day-old outgrowth, there are areas with many mitoses and areas with relatively few, perhaps corresponding to regions of the epidermis *in vivo* having different proliferative capacity. Although the existence of a population of basal cells outside the proliferative pool has not actually been determined *in vivo*, studies of other investigators suggest that this may be the case. Following wounding of the epidermis in man, there is a 48-hour delay before a rise in mitosis occurs (Sullivan and Epstein, 1963). This is similar to the situation in other tissues comprising cells in G_0 where, following an appropriate stimulus, DNA synthesis and mitosis take place after a variable lag period (Baserga, 1968, 1969).

From the currently available data it is thought that the epidermal hyperplasia of psoriasis results from both an increase in the size of the germinal population and a significant shortening of the cell cycle. Since the results of the present experiments suggest that some of the assumptions used in calculating the normal epidermal cell cycle may not be valid, it is relevant to ask whether conclusions about differences from the psoriatic cell cycle are indeed valid. It becomes imperative, therefore, to reinvestigate the characteristics of the cell cycle in normal human epidermis.

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COMBINED SURGICAL AND CHEMOTHERAPEUTIC TREATMENT OF FORTNER'S AMELANOTIC HAMSTER MELANOMA AMEL 3

E. HANEKE, W. WOHLRAB AND J. PEKER

Summary.—Golden hamsters were injected subcutaneously with melanoma cells and treated with vincaleucoblastine (VLB) after growth of the tumour. The prolongation of survival time after surgical removal of the tumour was compared with that of a non-operated, VLB-treated control group. The survival time of the operated hamsters was significantly prolonged. Although they did not show signs of tumour recurrence, 3 operated animals died from the VLB treatment. Comparing the results of both chemotherapy and combined chemotherapeutic and surgical treatment with those of experiments without cytostatic therapy, the markedly unfavourable effect of VLB on hamsters with melanomata is apparent. We suppose the immunosuppressive side-effect of VLB to be responsible for this negative result.

ALTHOUGH many studies on the treatment of human malignant melanoma have been reported during the last few years, the results are not satisfactory (see review by Marculescu, 1969). The effectiveness of various therapeutic regimes has been investigated in suitable models. Fortner's spontaneous transplantable hamster melanomata (Fortner, 1957) have been proved to be most useful, since—as for example the amelanotic melanoma AMel3—they are very similar to human melanomata with regard to their biological characteristics (Fortner *et al.*, 1961). The relatively modest results of a purely surgical treatment of the AMel 3 melanoma are comparable to the results in human melanomata (Haneke *et al.*, in press). Similarly a variety of different chemotherapeutic agents have not improved the unfavourable prognosis.

During the last decade vincaleucoblastine (VLB) has proved to be effective against several malignant diseases, especially when used in combination with other cytostatics ("polychemotherapy") (Gmachl, 1969). VLB is an alkaloid from *Vinca rosea*, L. which in low doses arrests mitosis in metaphase. In greater concentrations it exerts a strong cytostatic effect and inhibits protein biosynthesis (Fusenig and Obrecht, 1969; Lettré, 1969; Savel, 1966; Warnecke and Seeber, 1968).

The results of VLB treatment in human malignant melanomata are contradictory. While Bond and co-workers (1966) reported encouraging effects in some melanoma patients, most other authors have not observed good results (Braun-Falco and Burg, 1970; Newton, 1969; Vaitkevicius, 1969). The examination of the effectiveness of combined surgical and VLB treatment, with the aid of the AMel 3 hamster melanoma as a model, appeared desirable to us. Investigations are also in progress to study the influence on VLB effects of vitamin A (Hoefer-Janker *et al.*, 1969) and other lysosome labilizers.

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MATERIAL AND METHODS

Thirty strain Z 3 golden hamsters of both sexes, aged 4 to 6 months and having an average weight of 100 g were used. As recently described in detail elsewhere (Wohlrab and Peker, 1971; Haneke *et al.*, in press), the animals were injected with 2 × 10^6 viable AMel 3 cells subcutaneously into the right side of the body. Four days after inoculation, the tumours (mostly larger than peas) of 15 animals were removed by radical surgery (Table I). Together with the narcosis (0·02 mg atropine, 2 mg promazine intramuscularly and 150 mg urethan intraperitoneally) the hamsters received 0·05 mg to 0·08 mg VLB (Vinblastin, Richter Budapest/Hungary) intraperitoneally, then 0·1 mg VLB i.p. once a week. All non-operated control animals were given the same VLB doses at identical intervals. VLB administration was continued until the death of the animals, although the general condition of some hamsters was so serious that the last VLB injection had to be omitted. All animals were examined daily for tumour growth and recurrences respectively.

TABLE I.—*Correlation of Extent of Operation, Total VLB Dose (mg), Time of Onset of Metastasis (d post op.), Survival Period (d post op.) and Magnitude of Tumour Recurrence at the Time of the Animals' Spontaneous Death*

Extent of operation	Tumour size on op. day	Total VLB dose (mg)	Onset of metastases (d post op)	Survival time (d)	Magnitude of the tumour recurrence
Tumour excision with skin and fasciae	Lentil-sized	0·48	16	25	Plum-sized
Tumour excision with skin and fasciae	Pea-sized	0·28	7	15	Over plum-sized
Tumour excision with skin and fasciae	Lentil-sized	0·38	10	23	Over plum-sized
Excision with skin, fasciae and abdominal muscles	Over pea-sized	0·26	9	17	Nearly plum-sized
Excision with skin, fasciae and abdominal muscles	Nearly pea-sized	0·28	14	16	Pea-sized
Excision with skin, fasciae and abdominal muscles	Over pea-sized	0·48	9	30	Double plum-sized
Excision with skin, fasciae and abdominal muscles	Pea-sized	0·26	8	22	Plum-sized
Excision with skin, fasciae and thoracic muscles	Pea-sized	0·38	8	21	Over cherry-sized
Excision with skin, fasciae and thoracic muscles	Pea-sized	0·48	7	29	Over apple-sized
Excision with skin and fasciae, cleaning of the inguinal region	Double pea-sized	0·38	—	41*	—
Excision with skin and fasciae, cleaning of the inguinal region	Pea-sized	0·38	—	23*	—
Skeletonization of the left chest side	Pea-sized	0·26	7	22	Plum-sized
Skeletonization of the left chest side	Double pea-sized	0·16	8	14	Double cherry-sized
Excision with skin, fasciae, abdominal muscles and peritoneum	Over pea-sized	0·38	?	28†	?
Excision with skin, fasciae, abdominal and thoracic muscles and peritoneum	Over pea-sized	0·46	7	32	Double plum-sized

* Death certainly. † Death probably not due to tumour.

RESULTS

The effect of combined and purely chemotherapeutic treatment was judged by the tumour growth and the time of survival of the animals. The evaluation of survival time was made with the day of operation as the hypothetical time of diagnosis, the operation being usually 4 days after transplantation. All 15 operated hamsters survived the major surgical operation well. The wound healed without complication.

There were palpable recurrences or metastases in 12 animals after an average of 8 (7–16) days following operation. The remaining 3 hamsters did not develop signs of metastasis until death. In 2 animals the absence of tumours was proven histologically. They probably did not die from the tumour, but from the VLB treatment.

The VLB-treated, operated animals survived for 23·9 ± 7·3 (14–41) days from the day of operation. The VLB-treated control animals died after only 14·4 ± 4·4 (9–24) days. These survival times are shorter than in 2 comparable series without chemotherapy, each of 15 untreated controls (17·1 ± 6·5 days) and radically operated hamsters (28·7 ± 10·4 days) (Haneke *et al.*, in press). Between these 2 series without chemotherapy there was a statistically significant difference with regard to the life-prolonging effect of the surgical operation. The longer survival of operated animals is also evident in VLB-treated, operated animals (*t*-test: $\alpha = 0{\cdot}1$). In this series there is no indication of a lasting effect of the combined chemotherapeutic and surgical treatment. On the contrary, more rapid tumour growth was observed in VLB-treated animals. This was evident for tumours of non-operated controls, in which the melanomata after VLB were greater by about 50–100% at the time of spontaneous death, as well as for the recurrences after operation, which occured after comparable intervals, but clearly grew into larger tumours (Table I).

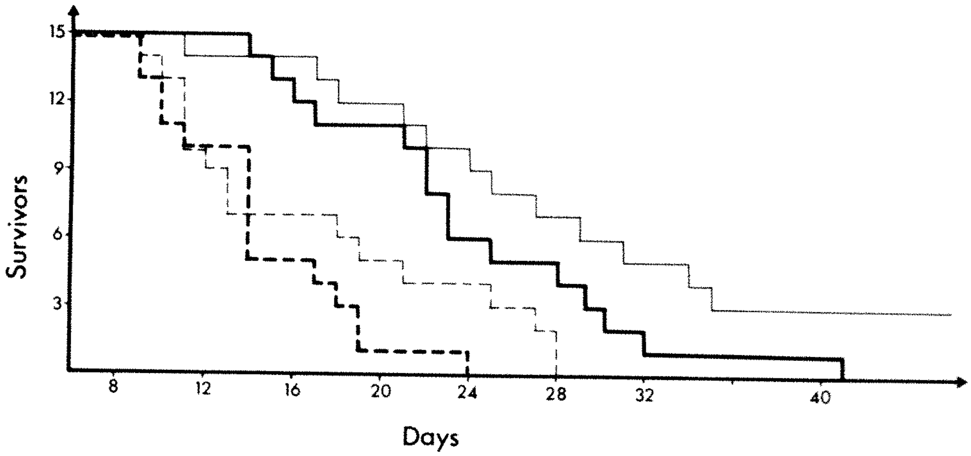

FIG. 1.—Effect of various therapy regimes on survival time. —— VLB-treated and operated; – – VLB-treated control animals; ——Operated hamsters; - - No therapy.

DISCUSSION

The therapy of human malignant melanoma is unsatisfactory. In general, the best results are to be expected from surgical treatment. To improve these results a combination with chemotherapy has been used repeatedly, although cytostatic therapy alone is known to accelerate the fatal course of human melanomata.

Previous studies on amelanotic hamster melanoma AMel 3 showed similar results for surgical treatment to those on human melanoma (Haneke *et al.*, in press). Šalamon and Storck (1963) obtained a complete inhibition of inoculated AMel 3 tissue by daily intraperitoneal administration of 2 mg thio-tepa/kg bodyweight, but cyclophosphamide was ineffective. Our study showed that treatment of the hamsters with about 1 mg VLB/kg bodyweight once a week did not result in tumour inhibition, but in more rapid tumour growth and a shorter survival time. Moreover, the tumours became larger before spontaneous death than in animals not treated cytostatically. Thus this very unfavourable effect of chemotherapy on Fortner's melanoma corresponds to the clinical observation of human melanomata.

Acceleration of tumour growth by VLB treatment can be explained by the marked immunosuppressive action of VLB (Aisenberg, 1963; Aisenberg and Wilkes, 1964; Jacquillat *et al.*, 1969; Schwarzenberg, 1969). The antigenicity of transplantable tumours is known to decrease with increasing transplantation generations (Sjögren, 1965), and Šalamon and Storck (1963) were able to produce neither active nor passive immunity against the melanotic hamster melanoma MMel 1. In general, the antigenicity of human melanoma seems to be very low, since DNCB-melanoma homogenate conjugates did not induce antibody formation against melanomata (Stüttgen and Remy, 1971), although anti-melanoma antibodies could be demonstrated with the aid of indirect immunofluorescence (Muna *et al.*, 1969; Lewis *et al.*, 1969; Morton *et al.*, 1971). Hence it might be suggested that a non-specific activity of the immune system is also able to inhibit tumour growth. Attempts to stimulate the immune system (see review by Stüttgen and Remy, 1971) with Freund's adjuvant or by intratumoral vaccination appear to be justified because the cell-mediated immune response is often very markedly inhibited (Cormia, 1968). The VLB dosage used by us apparently impaired the host's own defence mechanism more than the tumour. Hence it follows that the risk of chemotherapeutic treatment of malignant tumours with low antigenicity is very great. This effect is also known for alkylating agents and antimetabolites (Schmähl, 1969). Thus there is a real need for a model to enable us to establish whether cytostatic therapy is more harmful to the tumour or the host's immune system.

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THE ADENOSINE TRIPHOSPHATE CONTENT OF GUINEA-PIG SKIN STORED AT −196C°

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Summary.—Storage of guinea-pig ear skin at −196°C causes damage such that the ATP content of the skin falls very rapidly on thawing. The fall in ATP content on thawing can be arrested by placing the skin on serum medium at 37°C. Cryoprotective treatment before freezing and incubation after thawing on a strongly buffered Krebs/Ringer phosphate medium containing glucose and glycerol also minimizes ATP loss.

The implications of these findings are discussed.

ALTHOUGH storage of mammalian skin at the temperature of liquid nitrogen is now well established as a satisfactory process (Berggren and Lehr, 1965; Cochrane, 1968; Bondoc and Burke, 1971), it is also known that cells and subcellular particles can be damaged in the process of cooling to very low temperatures (Greiff, 1960). Privitera *et al.* (1958) showed that oxidative phosphorylation could be uncoupled in mitochondrial suspensions subjected to freezing and storage at low temperatures. Skin uses both aerobic and anaerobic pathways for the production of adenosine triphosphate (ATP) (Cruickshank, 1965), and damage to the process of oxidative phosphorylation could result in less ATP being synthesized. Since ATP is the primary source of energy for the metabolic processes of most cells, shortage of ATP might result in a general loss of viability. In the present study the ATP concentration in skin subjected to a variety of treatments, including freezing and storage at −196°, has been used to assess damage caused to the tissue by these treatments, and to investigate ways in which the effects of such damage might be minimized.

MATERIALS AND METHODS

Split thickness skin was cut aseptically from the ears of guinea-pigs, as described by Cruickshank (1954), and weighed on a torsion balance. For measurements of the ATP content of fresh skin, the specimens were extracted with known volumes of 0·6 N perchloric acid by grinding in a glass tissue homogenizer. The homogenates were centrifuged and ATP estimated in the supernatants, using test kits supplied by Boehringer.

In essence the method uses the ATP of the sample in the reaction:

$$\text{3-phosphoglycerate} + \text{ATP} \rightleftharpoons \text{1,3-diphosphoglycerate} + \text{ADP},$$

the reaction being catalysed by the enzyme phosphoglycerate kinase and Mg^{2+}. The 1,3-diphosphoglycerate is converted to glyceraldehyde 3-phosphate in the reaction:

$$\text{1,3-diphosphoglycerate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{glyceraldehyde 3-phosphate} + \text{NAD}^+ + \text{phosphate}.$$

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This is catalysed by the enzyme glyceraldehyde 3-phosphate dehydrogenase. The oxidation of NADH (nicotinamide adenine dinucleotide, reduced form) to NAD$^+$ (nicotinamide adenine dinucleotide, oxidized form) which occurs in this reaction is accompanied by a reduction in the u.v. adsorption of the compound, and this is measured spectrophotometrically at a wavelength of 340 nm (glyceraldehyde 3-phosphate is continuously removed from the reaction mixture by an irreversible reaction with hydrazine).

Skin samples for freezing were collected on to solutions of 15% (v/v) glycerol (as cryoprotective agent), in either saline or nutrient medium, and allowed to remain on the solution for at least 30 min. Methods for the freezing, frozen storage and thawing of skin were as described by Cochrane (1968). Skin samples were placed on filter paper moistened with the pre-freezing solution, and sealed into polythene bags. The bags were packed in solid CO_2 for 90 min and then suspended over liquid nitrogen for 24 hours. The types of experiment carried out on skin samples after removal from the nitrogen storage container are summarized in Table I. The nutrient media used are described in

TABLE I.—*Summary of Experiments on ATP Levels in Skin after 24 hours Storage at −196°C*

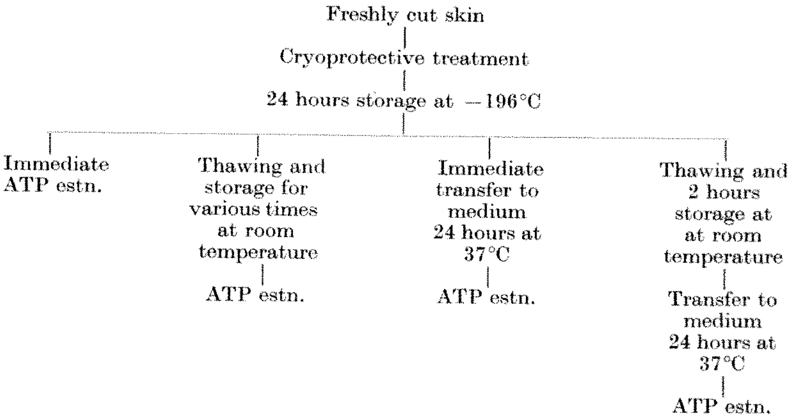
Freshly cut skin
Cryoprotective treatment
24 hours storage at −196°C
Immediate ATP estn.
Thawing and storage for various times at room temperature
ATP estn.
Immediate transfer to medium 24 hours at 37°C
ATP estn.
Thawing and 2 hours storage at at room temperature
Transfer to medium 24 hours at 37°C
ATP estn.

TABLE II.—*Media*

A. Based on the Krebs/Ringer phosphate solution described by Field (1948).

(i) Krebs/Ringer phosphate/glucose (KRP/G)

$$\left.\begin{array}{ll} 0\cdot154\ \text{mol/l NaCl} & 100\ \text{vols} \\ 0\cdot154\ \text{mol/l KCl} & 4\ \text{vols} \\ 0\cdot110\ \text{mol/l CaCl}_2 & 3\ \text{vols} \\ 0\cdot154\ \text{mol/l MgSO}_4.7\text{H}_2\text{O} & 1\ \text{vols} \end{array}\right\}\text{Soln. (a)}$$

$$\left.\left.\begin{array}{ll} 0\cdot25\ \text{mol/l Na}_2\text{HPO}_4 & 40\ \text{ml} \\ \quad\text{mol/l HCl} & 2\ \text{ml} \end{array}\right\} + \text{H}_2\text{O to 100 ml}\right\}\text{Soln. (b)}$$

(a) and (b) mixed in the ratio 10·8 : 2 : 1, final pH 7·4.
Glucose added to a concentration of 0·02 mol/l.
Streptomycin added to a concentration of 5 u/ml.

(ii) As (i), with the addition of adenosine to a final concentration of 0·0038 mol/l (Prankerd, 1956).

(iii) As (i), but with solutions (a) and (b) mixed in the ratio 1 : 2, i.e. the medium is no longer isotonic, but strongly buffered against lowering of pH.

(iv) Gomori phosphate buffer (Gomori, 1955)
0·067 mol/l KH_2PO_4 (a)
0·067 mol/l $Na_2HPO_4.2H_2O$ (b)
13·2 ml (a) + 86·8 ml (b) pH 7·6
Plus glucose to a final concentration of 0·02 mol/l.

B. Based on the serum medium described by Cruickshank and Lowbury (1952).

(i) Serum medium

homologous serum	5 vols
KRP	3 vols
5% glucose	1 vols } in 0·9% saline
Streptomycin (50 u/ml)	1 vols } in 0·9% saline

(ii) As (i), but serum replaced by serum ultrafiltrate.
(iii) As (i), but serum replaced by Physiogel (supplied by Swiss Red Cross).
(iv) As (i), but serum replaced by Plasma Protein Solution (Lister).

When the above media were used for cryoprotective treatment of skin before as well as for incubation after thawing, Analar glycerol was added to a final concentration of 15%. All solutions were sterilized before use by filtration through Millipore filters of pore size 0·22 μm.

Table II. Where an incubation on nutrient medium was involved, the sterility of the medium was checked at the end of the incubation period by plating out a sample of the medium on to a 4% blood agar plate and incubating overnight at 37°C.

RESULTS

The ATP content of fresh guinea-pig ear skin averaged 1·04 μmol/g. (The ATP content of serum medium was 0·01 μmol/ml and was disregarded). Incubation of fresh skin for 24 hours at 37°C on serum medium resulted in an apparent slight rise in ATP content (from 1·28 μmol/g to 1·51 μmol/g. not statistically significant).

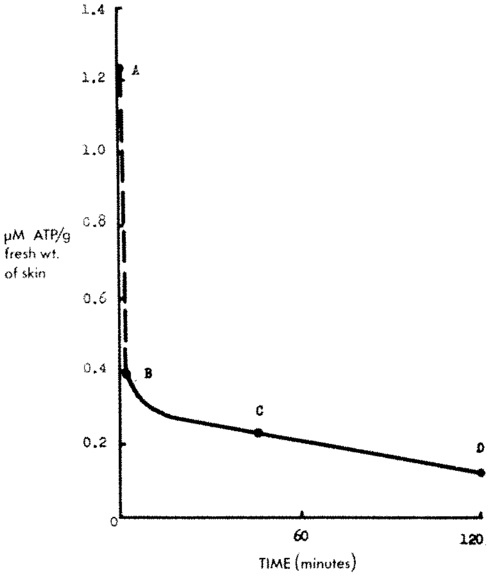

FIG. 1.—ATP content of guinea-pig ear skin stored at −196°C after thawing and storage at room temperature. A, Fresh skin; B, Skin stored 24 hours at −196 °C; C, Skin stored 24 hours at −196°C, 5 min at 37°C and 40 min at room temperature; D, Skin stored 24 hours at −196°C, 5 min at 37°C and 115 min at room temperature.

Variable ATP values were obtained when estimations were made on skin immediately after removal from frozen storage. The highest value, 1·11 μmol/g, was within the range for fresh skin. The data presented in Fig. 1 demonstrate a decline in ATP content with time in skin removed from storage over liquid nitrogen and subsequently kept at room temperature. Table III shows that the loss of ATP resulting from the 2-hour wait at room temperature was permanent

TABLE III.—*The Effect of 24 hours' Incubation at 37°C on Serum Medium on the ATP Content of Skin Kept at Room Temperature for 2 hours after Thawing from* −196°C

Sample no.	Treatment after removal from storage at −196°C	Mean μmol ATP/g F.W. ± S.E.M. (no. of expts.)	Statistical significance
1	None (immediate ATP estn.)	0·620±0·007 (3)	0·4 > p > 0·3 N.S.
2	Serum medium (immediately after removal from N_2)	0·500±0·106 (3)	
3	5 min 37°C, 120 min room temp. followed by ATP estn.	0·114±0·025 (3)	0·3 > p > 2 N.S.
4	As (3), then 24 hours incub. serum medium 37°C	0·198±0·038 (3)	

TABLE IV.—*ATP Levels in Skin Incubated on Various Media for 24 hours at 37°C after Freezing at* −196°C *on* 15% (*v/v*) *Glycerol in Saline*

Sample no.	Incubation medium	Mean μmol ATP/g F.W. ± S.E.M. (no. of expts.)
1	None (immediate ATP estn.)	0·326±0·072 (3)
2	Serum medium	0·520±0·036 (3)
3	Krebs/Ringer phosphate + glucose	0·069±0·019 (3)
4	Saline	0·037±0·005 (3)
5	Serum	1·096±0·072 (3)
6	Gomori phosphate buffer + glucose	0·092±0·009 (3)

to the extent that it was not corrected during subsequent incubation on a medium known to be effective in maintaining the ATP content of undamaged skin.

Table IV shows the ATP content of frozen skin after 24 hours incubation on various media, the transfer to the pre-warmed medium having been made with the least possible delay after removal from storage. Only serum medium was effective in maintaining the ATP content of the specimens.

TABLE V.—*ATP Levels in Skin Incubated for 24 hours on Various Media Containing* 15° (*v/v*) *Glycerol at 37°C after Freezing to* −196°C *on the Same Media*

Sample no.	Pre-freezing and incubating medium	Mean μmol ATP/g F.W. ± S.E.M. (no. of expts.)	Final pH
1	Serum medium	0·883±0·057 (3)	Not measured
2	Plasma protein soln.	0·100±0·011 (3)	Not measured
3	Krebs/Ringer phosphate + glucose	0·382±0·082 (3)	Less than 6·5
4	As 3, + adenosine	0·155±0·024 (3)	Less than 6·5
5	Physiogel medium	0·085±0·045 (3)	6·70
6	KRP/G, strongly buffered	0·618±0·076 (3)	6·70
7	Serum ultrafiltrate	0·444±0·076 (3)	6·67
8	Serum medium	0·726±0·256 (3)	6·65
9	Serum ultrafiltrate	0·295±0·049 (3)	6·62

An attempt was made to devise a biologically simpler medium which would be as effective as serum medium in maintaining ATP levels in skin after thawing from −196°C (Table V). The media selected consisted either of inorganic buffer and salt solutions with the addition of glucose, or were based on the serum medium described by Cruickshank and Lowbury (1952), the serum component being replaced by simpler solutions. The media all contained 15% (v/v) glycerol and were used for cryoprotective treatment prior to freezing, as well as incubation immediately on removal from frozen storage. Comparison of the ATP values of skin samples incubated on serum medium without glycerol (Tables III and IV) and with glycerol (Table V) suggests that the presence of glycerol in the incubation medium was without harmful effect. Maintenance of pH has been thought to be of importance for cell survival (Ceccarini and Eagle, 1971). In conformity with this, the strongly buffered Krebs/Ringer phosphate + glucose medium was the most efficient in conserving ATP. However, as Table VI shows, the presence of the medium during freezing did not protect against the loss of ATP occurring as a result of storage at room temperature after thawing, although the pH values after incubation were 6·87 and 7·05 respectively.

Table VI.—*ATP Levels in Skin Given Cryoprotective Treatment with Glycerinated Media before Freezing, Maintained for 2 hours at Room Temperature after Thawing from −196°C and Incubated at 37°C*

Sample no.	Incubation medium	Mean μmol ATP/g F.W. ± S.E.M. (no. of expts.)	Final pH
1	None (immediate ATP estn.)	1·112±0·007 (3)	Not measured
2	Krebs/Ringer phosphate + glucose	0·084±0·023 (3)	6·87
3	Serum medium	0·125±0·032 (3)	7·05

DISCUSSION

In damaged cells, ATP appears to be very labile. This is not unexpected, since ATP can be synthesized in several ways and used or destroyed in many more. In particular, the amount of ATP found in skin immediately after removal from storage at −196°C ranged from the value found in fresh skin to about one-third of the value. This could be due to destruction of ATP occurring at a very high rate during thawing, and to unavoidable variations in the time taken to extract ATP from the tissue. In addition, there may be variation in the amount of damage caused by the freezing process, possibly depending on biological variation among the specimens themselves.

However, it is the ability of skin to maintain or re-establish its ATP content which is of practical importance. The ATP content of skin stored frozen and subsequently incubated at 37°C for 24 hours on serum medium was consistently above 0·52 μmol/g, except when the specimens were allowed to wait at room temperature after thawing and before being incubated on the medium. In the latter case a rise in ATP concentration was observed, but the level attained after incubation (0·20 μmol/g) did not approach the normal range, and the difference in ATP levels immediately after the wait at room temperature, and after a further 24 hours incubation at 37°C on serum medium, was not statistically significant. There seems to be a time lag before the serum medium influences the ATP level of the specimen, as skin incubated on serum medium immediately after removal from frozen storage declined slightly in ATP content, but not to the levels found in skin kept at room temperature.

Attempts were made to find a simple, clinically acceptable medium which could be used for treatment of the skin prior to freezing, and which would minimize the ATP loss occurring at room temperature after thawing.

A buffered medium is necessary for the incubation of undamaged skin to compensate for the production of lactic acid (Cruickshank and Trotter, 1956).

Media in which Lister Plasma Protein Solution or Physiogel were used in place of serum were ineffective. Gomori phosphate buffer at pH 7·6, to counteract the drop in pH observed with saline alone or standard KRP/G medium, was also ineffective. KRP/G was ineffective when it was supplied only after thawing, but partly effective when present in the pre-freezing medium. The addition of adenosine to the KRP/G medium made no significant difference. The presence of serum ultrafiltrate in the medium for cryoprotection and/or incubation was partly effective. The best results were obtained when the buffering capacity of the KRP/G was increased in order to maintain the pH of the medium at a physiologically acceptable level during incubation. The higher buffering capacity required in medium for frozen skin suggests that acid products are released in large quantities. When strongly buffered KRP/G medium, containing 15% (v/v) glycerol, was used both for cryoprotective treatment and incubation, the ATP levels observed were comparable with those of skin frozen on a glycerol in saline solution and transferred to serum medium when removed from storage.

However, neither serum medium nor KRP/G medium protected skin stored at −196°C against ATP loss during storage at room temperature. It therefore seems advisable that there should be as little delay as possible between thawing stored skin and placing it upon a recipient site, especially when the best possible graft take is required, as with autograft skin. If a delay after thawing becomes inevitable, it might be helpful to open the bags, add warm sterile KRP/G medium, and incubate at 37°C until the skin is required.

There is a clear parallel between the environmental conditions of skin placed on serum medium at 37°C and skin placed on a suitable recipient site during grafting. The graft bed would provide the better conditions, since there is replacement of medium leading to good nutrition of the cells of the graft, and maintenance of pH (Smahel, 1971). This may be the reason for the good results obtained clinically in grafting skin stored at −196°C, although poorer results might perhaps be expected when stored grafts are used on sites prepared by tangential excision (Janzekowic, 1968; Groves and Lawrence, 1971), where the graft bed might provide less adequate nutrition and buffering for the graft.

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Pharmacology and Treatment

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THE EFFECT OF GLYCEROL ON THE PERCUTANEOUS ABSORPTION OF METHYL NICOTINATE

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Summary.—The effect of glycerol on the rate of percutaneous absorption of methyl nicotinate from aqueous vehicles was investigated. The partition coefficients of methyl nicotinate between isopropyl myristate and aqueous solutions of glycerol were determined. The diffusivity of methyl nicotinate in various aqueous solutions of glycerol was also studied. It was shown that addition of glycerol in concentrations above 60% delayed the onset of erythema in human skin. The results suggest that this effect is related to the partition coefficient and diffusivity of methyl nicotinate in the aqueous vehicle. The osmotic effects of glycerol are negligible.

GLYCEROL is commonly used as an ingredient of dermatological vehicles. It has humectant properties which provide an adherent film for the application of powders to the skin surface; in higher concentrations it has preservative properties, and it prevents evaporation of the aqueous phase of creams on storage. Some of these features add to the cosmetic acceptability of the preparation. It has long been known that glycerol reduces the caustic properties of phenol on the skin, and for this reason it is an essential ingredient of phenol ear drops.

Barrett *et al.* (1964) have shown that the addition of glycerol to aqueous cream delays the onset of erythema produced by methyl nicotinate, and it was suggested that this was possibly due to an osmotic effect. However, other interpretations of this effect are possible, and this investigation was undertaken to elucidate this problem. Simple aqueous solutions of methyl nicotinate containing increasing concentrations of glycerol were used to assess the effect of glycerol on the time of onset of erythema on the skin of the forearm.

METHODS

Determination of Onset of Erythema

The experiments were carried out with solutions containing 0·5% methyl nicotinate. This was dissolved in water and in water containing increasing concentrations of glycerol, i.e. 10%, 40%, 60%, 80% and 100%. In order to avoid the difficulties caused by spreading and " running " on the skin surface, the solutions were applied using a modified technique. A disc of filter paper approximately 1 cm in diameter was made, using a cork borer. This was placed on the flexor aspect of the forearm, and 50 μl of the solution being tested was applied. In this way both running and spreading were kept to a minimum, and the onset of the erythema was recorded when a red halo was seen round the edge of the paper.

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The experiments were carried out on 2 subjects. Six filter paper discs were placed on each arm and the 6 solutions applied. The sites were labelled 1 to 12 and the solutions 1 to 6. Solution 1 was placed on sites 1 and 2, solution 2 on sites 3 and 4, and so on. The solutions were then rotated for a complete cycle so that 24 results were obtained for each solution. The experiments were carried at 25°C ± 1°C and at a relative humidity of approximately 45%. The results are recorded in Table I.

To determine the effect of changes in osmotic pressure in the vehicle, an exactly similar technique was used to determine the onset of erythema produced by aqueous solutions of 0·5% methyl nicotinate with increasing concentrations of sodium chloride (0·9%, 1·8%, 2·7% and 3·6%). The results are shown in Table II.

Determination of Partition Coefficients

The partition coefficients were determined using isopropyl myristate as the lipid phase. This was selected because there is a high proportion of monoesters in the skin lipids and it has for this reason been previously used in the investigation of percutaneous absorption (Poulsen *et al.*, 1968).

Solutions (0·5%) of methyl nicotinate were made with water and with increasing glycerol concentrations (10%, 40%, 60%, 80%, 100%); 10 ml of each of these solutions was then placed in a closed container with 10 ml of isopropyl myristate and shaken mechanically for 24 hours. After this period the 2 layers were allowed to separate, 0·5 ml samples were taken and the concentration of methyl nicotinate determined by u.v. spectrophotometry. Methyl nicotinate in ethanol produces an absorption peak at 263 nm, a 1% solution having an optical density of 206. The samples were diluted 1 : 100 in ethanol, the optical density determined and the concentration of methyl nicotinate calculated. Samples were taken from both layers, and the experiment was repeated 3 times at 24°C.

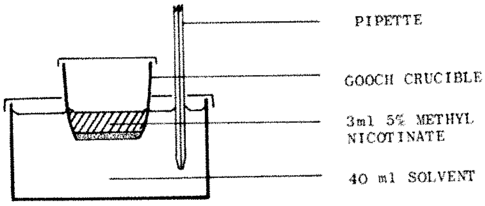

FIG. 1.—Diagram of diffusion apparatus.

The partition coefficient was expressed as the ratio of the concentration of methyl nicotinate in isopropyl myristate to the concentration of methyl nicotinate in aqueous solution. The results are recorded in Table III.

Determination of Diffusivity

In a previous study (Baker *et al.*, 1969) diffusion studies were carried out using a simple non-membrane diffusion cell. Whilst this technique allowed comparisons to be made between different solvents, it had the disadvantage of introducing errors due to the effect of convection currents in the upper layer.

In this investigation diffusion was studied using a porous porcelain barrier. The diffusion cell consisted of a porcelain crucible with a porous bottom, as illustrated in Fig. 1. In order to remove air from the porous barrier, some of the solvent was first drawn through the crucible under reduced pressure, thus causing saturation of the barrier with the solvent. The crucible was then placed in a beaker containing 40 ml of solvent, and 3 ml of a 5% solution of methyl nicotinate in the solvent was introduced into the crucible. To eliminate differences due to hydrostatic pressure, the levels of the solution inside the crucible and the surrounding solvent were the same. One ml samples were taken from the solvent, diluted to 100 ml with water, and the concentration of methyl nicotinate determined by u.v. spectrophotometry.

RESULTS

The results in Table I confirm that the onset of erythema is appreciably delayed by the addition of glycerol in concentrations over 60%. There is close correlation between the results in this study and those previously reported by Barrett *et al.* (1964). In the earlier study aqueous cream B.P. was used as the vehicle, and this contains 70% water. In the present investigation simple aqueous solutions were used. If the glycerol concentrations in the aqueous phase of the cream are compared with the glycerol concentrations in the aqueous solutions, there is good

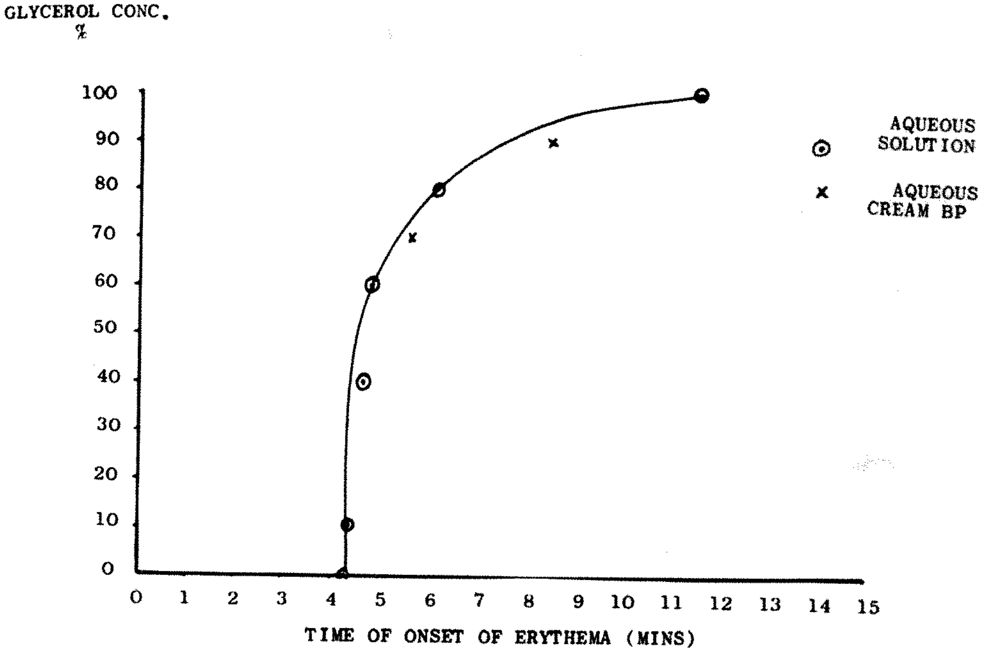

Fig. 2.—Graph showing onset of erythema at different concentrations of glycerol in aqueous solution (○——○).

Note: For comparison, the values for aqueous cream had been taken from the paper by Barrett *et al.* (1964) and expressed as the concentration of glycerol in the aqueous phase of the cream.

Table I

Glycerol concentration (percentage)	Mean time of onset of erythema (min)	Standard deviation (min)
0	4·29	0·61
10	4·42	0·61
40	4·73	1·37
60	4·82	0·61
80	6·17	2·21
100	11·53	3·19

agreement in the onset of erythema, as shown in Fig. 2. That the delay in the onset of erythema was not due to an osmotic effect in the vehicle was shown by the results in Table II, in which there was no delay in the onset of erythema with increasing concentrations of sodium chloride.

TABLE II

Sodium chloride (%)	Mean time of onset of erythema (min)	Standard deviation
0·9	3·85	0·138
1·8	3·84	0·073
2·7	4·12	0·053
3·6	3·88	0·124

TABLE III

Glycerol concentration (%)	Methyl nicotinate average % in IPM	Partition coefficient
0	0·383	3·28
10	0·379	3·13
40	0·376	3·05
60	0·367	2·76
80	0·348	2·26
100	0·258	1·07

The results in Table III show that, with increasing concentrations of glycerol, there is a reduction in the partition coefficient between isopropyl myristate and methyl nicotinate in aqueous solution. The methyl nicotinate becomes less available for penetrating the skin as the concentration of glycerol is increased.

Methyl nicotinate rapidly penetrates the skin to produce a response within minutes of application. In these circumstances the diffusivity of the methyl nicotinate could have an appreciable effect on the rate of onset of erythema. In order to estimate this effect, the rate of diffusion of methyl nicotinate in the different vehicles was determined and the relative diffusivities calculated using Fick's 1st Law. This can be expressed as follows:

$$\frac{dc}{dt} = -DA\frac{dc}{dx} \qquad (1)$$

where c = concentration of diffusing substance at time t
x = thickness of membrane
D = diffusivity
A = area of membrane.

From equation (1), it can be shown that:

$$\log \frac{C_\infty}{C_\infty - C_1} = kt$$

where C_∞ = concentration of diffusing substance at equilibrium
C_1 = concentration of methyl nicotinate in lower compartment at time t
$k = \frac{DA}{x}\left(\frac{1}{V} + \frac{1}{V_1}\right)$

and V, V_1 are respectively the volumes in the upper and lower compartments.

Under the conditions of the experiment, A, x, V and V_1 are all kept constant; thus k is proportional to D, the diffusivity. Figure 3 shows a straight-line relationship when $\log (C_\infty/C_\infty - C_1)$ is plotted against time, and the slopes of the graphs provide a measure of the diffusivity of methyl nicotinate in the different vehicles. From these data the relative diffusivities in Table II were derived, taking the diffusivity of methyl nicotinate in glycerol as unity.

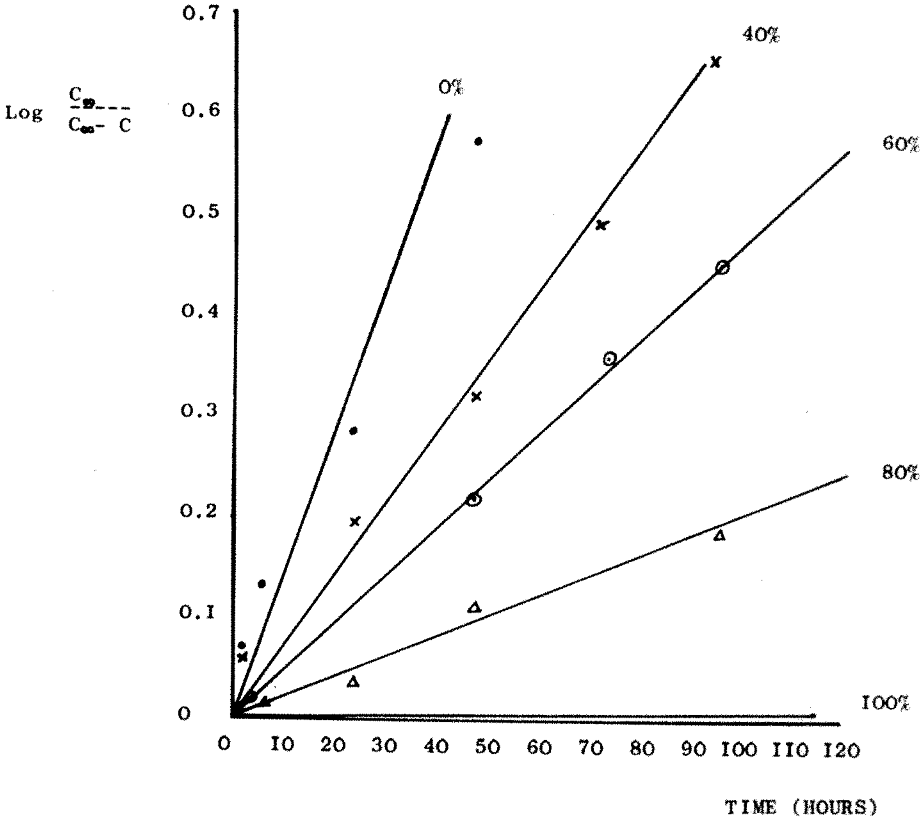

FIG. 3.—Graph showing the relationship of diffusion of methyl nicotinate in glycerol solution at different times (see text).

TABLE IV

Glycerol (%)	Slope of graph	Relative diffusivity
0	0·0270	270
40	0·0134	134
60	0·0089	89
80	0·00385	38·5
100	0·0001	1·0

DISCUSSION

This study has shown that, in simple aqueous systems, increasing concentrations of glycerol appreciably delay the penetration of methyl nicotinate into the skin. The osmotic effects of the vehicle have been shown to be negligible by the results obtained with similar aqueous solutions containing increasing concentrations of sodium chloride. These results suggest that methyl nicotinate penetrates the skin via a lipid rather than an aqueous pathway.

The altered partition coefficient and diffusivity are of greater importance than the change of osmotic pressure of the vehicle. The partition of methyl nicotinate between isopropyl myristate and aqueous solutions of glycerol is strikingly reduced when the glycerol concentration approaches 60%. It seems probable that this is the major factor in reducing the availability of the methyl nicotinate to penetrate the skin. As the partition coefficient is reduced, the time of onset of erythema is increased. The relationship is shown in Table V, in which the products of the times of onset of erythema and the partition coefficients have been calculated. Bearing in mind the standard deviations for the times of onset of erythema, the values are remarkably constant, suggesting that there is an inverse relationship between the two for this series of vehicles.

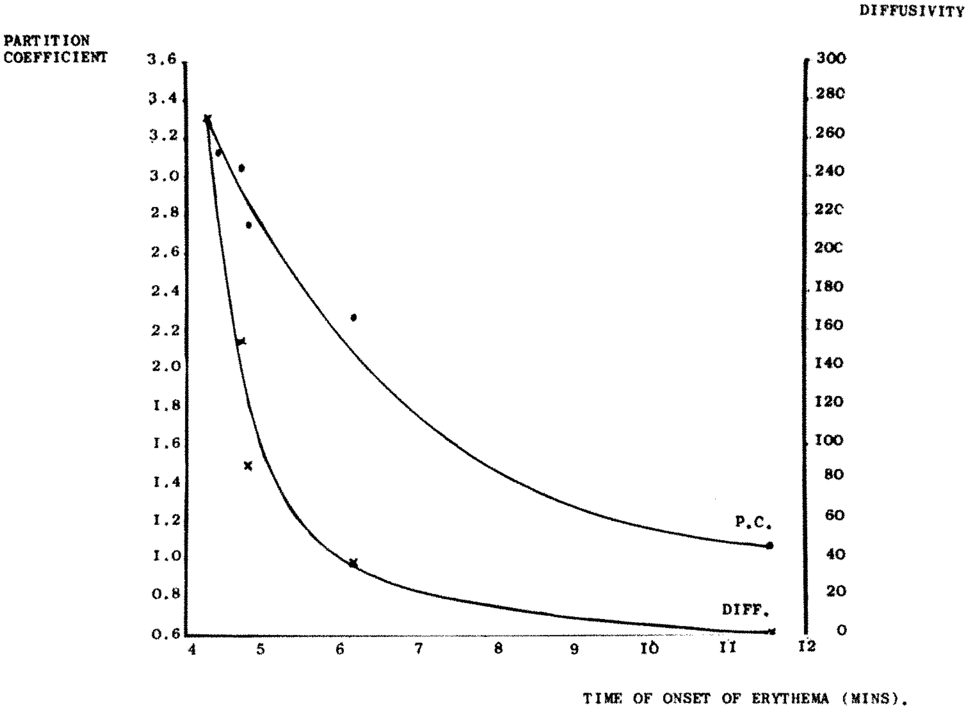

FIG. 4.—Graph showing similarity of relationship of onset of erythema to both the partition coefficient and diffusivity.

The importance of partition coefficients has previously been stressed by Katz and Shaikh (1965) and by Teorell (1969). Higuchi (1960) suggested a mathematical expression relating the rate of absorption to the partition coefficient and diffusivities of the substance in the skin and in the vehicle. More recently, Ostrenga *et al.* (1971) have shown that the partition coefficients of fluocinolone and fluocinonide are critical factors in determining their *in vivo* activity when formulated in aqueous propylene glycol gels.

TABLE V

Glycerol concentration (percentage)	Partition coefficient (P.C.)	Mean time of onset of erythema (t)	P.C. × t
0	3·28	4·29	14·1
10	3·13	4·42	13·8
40	3·05	4·73	14·4
60	2·76	4·82	13·3
80	2·27	6·17	14·0
100	1·07	11·53	12·4

The rate of diffusion of methyl nicotinate in the vehicle has been shown to influence its duration of action on the skin by Fountain *et al.* (1969). It seems possible that diffusivity may also influence the rate of onset of erythema, which will depend upon the number of molecules making an impact with unit area of skin surface in unit time. By reducing molecular movement of the methyl nicotinate in the vehicle, a lowered diffusivity will reduce the number of molecular impacts and retard the rate of onset of erythema. The composite graph (Fig. 4) shows that the rate of onset of erythema follows a course similar to both the partition coefficient and the diffusivity. It seems probable, therefore, that the rate of onset of erythema is related to the partition coefficient and the diffusivity. Lowered diffusivity reduces the number of molecular impacts, and lowered partition coefficient reduces the number of methyl nicotinate molecules retained by the skin.

The results in this study are closely in accordance with those obtained by Barrett *et al.* (1964) for aqueous creams containing glycerol. This suggests that the aqueous phase of the cream acts on the skin surface independently of the dispersed oily phase. This, however, will be true only if the partition coefficient of the methyl nicotinate between the aqueous and oily phases of the cream is strongly in favour of the former. If the oily phase is also capable of dissolving the methyl nicotinate, it may compete with the skin for the methyl nicotinate and also affect the rate of onset of erythema.

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THE EFFECT OF CLINDAMYCIN IN ACNE—A CLINICAL AND LABORATORY INVESTIGATION

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Summary.—Skin surface lipid composition and sebum excretion rate were determined before and after one and 2 months of therapy in patients taking clindamycin for severe acne vulgaris. All patients showed marked clinical improvement. Clindamycin produced a significant reduction in skin surface free fatty acids and a significant reciprocal increase in skin surface triglycerides after one and 2 months of therapy. There was no significant change in the sebum excretion rate. These results support the hypothesis that free fatty acids are important in the pathogenesis of acne vulgaris.

ALTHOUGH most patients with acne vulgaris respond satisfactorily to oral tetracycline (Lane and Williamson, 1969), there is a group of patients who fail to respond (Cunliffe and Cotterill, 1970). Thus more effective therapy, especially for the severe forms of acne vulgaris, is required. Lincomycin has proved effective, especially in cystic acne (Hall *et al.*, 1968), and recently a better-absorbed synthetic derivative of this antibiotic clindamycin (7*(S)chloro-7-deoxylincomycin) has become available. It has recently been suggested that this drug may have a place in the therapy of cystic acne (Ashton *et al.*, 1970)

We assessed the effect of this drug in acne and simultaneously investigated both the skin surface lipid composition and sebum excretion rate, which are thought to be of importance in the pathogenesis of this condition (Freinkel *et al.*, 1965; Strauss and Pochi, 1965; Cunliffe and Shuster, 1969a).

PATIENTS AND METHODS

Twenty-one patients, 14 males and 7 females with severe acne vulgaris, were studied; their ages ranged from 14–22 years (mean 18 years). Four patients had failed to respond to adequate doses of tetracycline, and 2 further patients had failed to respond to either tetracycline or a trimethoprim–sulphamethoxazole combination.

At the patients' first and subsequent attendances the acne was assessed clinically and the sebum excretion rate was determined, using absorbent papers on the forehead by the method of Strauss and Pochi (1961), modified by Cunliffe and Shuster (1969b). Skin surface lipid composition was determined semi-quantitatively by thin-layer chromatography (Cotterill *et al.*, 1971a). All collections were made at the same time of day to avoid any circadian variations in sebum excretion rate (Burton *et al.*, 1970) or skin surface lipid composition (Cotterill *et al.*, 1971b). No topical, u.v. light or systemic therapy was given before or during the investigation. Therapy consisted of 150 mg clindamycin hydrochloride daily for 2 months, and patients were assessed at 4-weekly intervals.

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* Available as Dalacin C—Cleocin in U.S.A. (Upjohn).

RESULTS

There was a considerable improvement in the acne vulgaris at both the first and second attendance in all patients. Many subjects commented spontaneously that they thought their acne began to improve within 2 weeks of taking the drug. The results for the sebum excretion rate and skin surface lipid composition with probabilities are shown in Tables I and II.

TABLE I.—*Showing Sebum Excretion Rate and Skin Surface Lipid Composition Before and After One and 2 Months of Therapy with Clindamycin*

Subject	1	2	3	4	5	6	7
Sebum excretion rate (μg/cm^{-2}/min)	1·52	0·95	1·20	0·68	1·65	1·08	0·82
	1·30	1·11	0·91	0·74	1·56	0·75	0·66
						0·89	0·79
Squalene (%)	16·8	16·3	13·7	15·1	14·1	13·7	16·4
	16·1	14·8	15·4	16·2	13·1	12·4	14·7
	15.4					10·8	9·6
Wax esters (%)	34·8	29·2	29·8	32·5	34·1	27·5	27·9
	31·5	33·9	30·3	32·0	28·1	25·9	26·8
	21.8					33·6	23·6
Triglycerides (%)	47·8	44·7	55·2	49·0	45·2	42·1	49·8
	52·4	45·9	53·2	50·8	49·1	59·4	52·3
	62·4					43·4	58·7
Free fatty acids (%)	0·50	9·4	2·1	3·1	6·4	16·1	5·5
	0·0	4·7	1·1	1·0	8·7	2·1	5·8
	0·2					1·6	7·1

Subject	8	9	10	11	12	13	14
Sebum excretion rate (μg/cm^{-2}/min	1·79	0·89	0·95	0·94	1·15	0·16	1·13
	1·56	0·98	0·97	1·05	1.54	0·56	0·58
	1·77	0·75	1·06	1·46	1·30	0·65	0·66
Squalene (%)	16·1	13·0	13·8	19·1	10·9	13·2	10·4
	12·6	14·2	13·3	13·1	13·4	14·7	11·2
	16·5	14·6	15·4	13·2	15·9	14·6	9·8
Wax esters (%)	30·3	31·2	25·8	20·6	30·6	24·4	29·0
	26·8	29·5	27·1	25·1	32·9	27·8	25·0
	28·9	29·9	28·8	21·6	27·9	29·5	24·1
Triglycerides (%)	52·7	46·2	56·8	48·5	46·3	58·5	57·3
	57·2	55·5	59·0	45·9	52·8	52·3	60·0
	28·9	47·7	45·6	54·1	50·6	55·5	62·5
Free fatty acid (%)	0·7	9·4	3·4	11·5	13·2	3·4	1·8
	3·0	0·7	0·5	13·2	0·7	3·9	2·6
	0·0	7·5	9·2	10·3	4·9	0·0	2·1

Subject	15	16	17	18	19	20	21
Sebum excretion rate (μg/cm^{-2}/min	1·16	1·57	1·38	1·06	1·25	1·58	1·50
	0.92	1.36	1.10	1.03	1.38		
	1·20		1·01		1·06	1·32	1·71
Squalene (%)	16·7	13·7	12·6	16·5	13·2	15·1	11·0
	11·2	10·9	10·0	15·7	13·3		
	9·7		14·6		13·8	15·3	12·1
Wax esters (%)	21·8	30·4	24·6	26·2	26·8	29·7	27·0
	20·8	21·3	23·3	27·7	30·8		
	21·4		23·1		24·9	29·0	34·0
Triglycerides (%)	49·0	53·5	45·7	47·9	55·9	55·1	26·0
	63·4	63·4	50·0	51·2	55·0		
	65·9		56·1		60·3	55·5	49·1
Free fatty acids (%)	11·9	2·3	17·0	8·8	4·0	0·0	34·0
	4·3	4·2	16·4	4·4	0·2		
	2·8		6·1		0·8	0·0	4·8

TABLE II.—*Showing Probabilities for Changes in Sebum Excretion Rate and Skin Surface Lipid Composition After One and 2 Months of Therapy with Clindamycin*

Duration of therapy (month)		Sebum excretion rate	Squalene (%)	Wax ester (%)	Triglyceride (%)	Free fatty acids (%)
1	*n*	18	19	19	19	19
	t	1·68	1·92	0·17	3·34	2·50
	2*P*	$>0{\cdot}1$	>0.1	$>0{\cdot}8$	$<0{\cdot}005$	$<0{\cdot}025$
2	*n*	15	16	16	16	16
	t	0·48	0·59	0·53	3·00	2·34
	2*P*	$>0{\cdot}6$	$>0{\cdot}5$	$>0{\cdot}5$	$<0{\cdot}01$	$<0{\cdot}05$

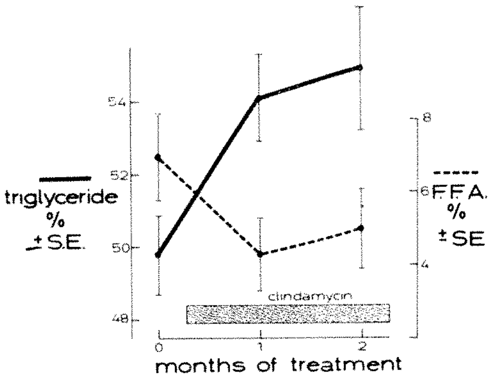

FIG. 1.—Showing the effects of clindamycin on the percentages of skin surface lipid, free fatty acids and triglyceride after one and 2 months of therapy.

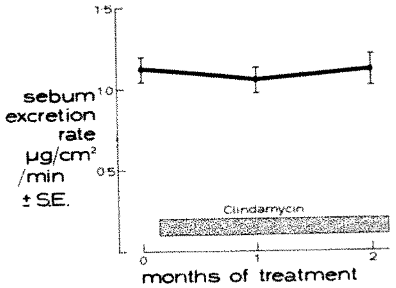

FIG. 2.—Showing the effects of clindamycin on sebum excretion rate after one and 2 months of therapy.

Clindamycin produced a significant decrease in the percentage of skin surface free fatty acids after one and 2 months of therapy, and there was a significant corresponding inverse rise in sebum triglycerides (Fig. 1). There was no significant change in the sebum excretion rate (Fig. 2).

DISCUSSION

Several broad spectrum antibiotics such as tetracycline (Lane and Williamson, 1969) and a trimethoprim–sulphonamide combination (Cotterill *et al.*, 1971c) are effective in acne vulgaris, and clinical improvement is associated with a decrease in skin surface free fatty acids and a corresponding increase in skin surface triglyceride (Freinkel *et al.*, 1965; Cotterill *et al.*, 1971c). However, not all patients respond satisfactorily to tetracycline or to a trimethoprim–sulphamethoxazole combination, and effective alternative therapy is required. This failure to respond may be due to defective absorption of tetracycline, and it is known that the absorption of this antimicrobic can be impaired by food (Kirby *et al.*, 1961). It is also possible that tetracycline fails to reach the diseased pilosebaceous unit in some patients, despite normal absorption. Alternatively, *Corynebacterium acnes*, which is probably the most important bacterium involved in the development of acne, may have become resistant to this antibiotic.

Clindamycin produced striking clinical improvement in our patients, all of whom had severe acne. Four of these patients had previously failed to respond clinically to tetracycline, and 2 had failed to respond to either tetracycline or to a trimethoprim–sulphamethoxazole combination.

The clinical improvement was associated with a significant decrease in the percentage of surface lipid free fatty acids. There was a corresponding increase in triglycerides, which form the substrate from which the free fatty acids are derived, probably chiefly by the lipolytic action of the *Corynebacterium acnes* (Freinkel and Shen, 1969).

Clindamycin has been demonstrated in sebum after oral therapy, and it is known that *Corynebacterium acnes* is sensitive to this antibiotic (Duke *et al.*, 1971). It is therefore tempting to attribute the changes we have found to inhibition of *Corynebacterium acnes*. The effectiveness of clindamycin in patients who have failed to respond to other antibiotics may also be a reflection either of its better absorption from the gastro-intestinal tract (McGehee *et al.*, 1968) or its greater lipid solubility (Whitmarsh, 1971).

We conclude that clindamycin, in a relatively small dose of 150 mg daily, provides effective therapy in patients with severe acne vulgaris and in those who have failed to respond to either tetracycline or a trimethoprim–sulphamethoxazole combination. The fact that this and other antibiotics effective in acne produce similar changes in the skin surface lipid composition supports the hypothesis that free fatty acids are important in the pathogenesis of acne vulgaris.

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TREATMENT OF PITYRIASIS RUBRA PILARIS WITH AZATHIOPRINE

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Summary.—Treatment with azathioprine has been used successfully in 5 cases of widespread PRP. Four cases cleared completely and in the other, after good improvement, treatment was abandoned on account of abdominal pains. Complete clearing may be slow, and treatment needs to be long continued. Two cases remain clear without treatment after 3 years and 2 years respectively. No serious side-effects have been seen with the doses used.

WIDESPREAD severe pityriasis rubra pilaris (PRP) is not common. It may be very distressing to the patient on account of limitation of movement caused by extreme thickening and fissuring of the skin of the palms and soles. Past methods of treatment, most commonly large doses of Vitamin A orally, and salicyclic acid creams, have been unsatisfactory.

Methotrexate has been used in doses similar to those used in psoriasis (Brown and Perry, 1966; Anderson, 1966). Good results have been reported, but in our experience this drug has not been very successful.

We first used azathioprine empirically when complications of oral corticosteroid therapy outweighed the limited improvement it had produced in a seriously affected patient.

Azathioprine is widely used for the suppression of immunological responses. It has proved to be a relatively safe drug which is effective in a wide range of auto-immune diseases, and has an established place in the management of renal transplantation. In many cases it makes possible a significant reduction in the dosage of corticosteroid.

During the past 4 years we have treated 5 cases of widespread erythrodermic PRP with azathioprine. In 4 cases there has been complete clearing. In the other, very good improvement occurred before the drug was stopped because of abdominal pains.

CASE REPORTS

Case 1.—J.M., a woman aged 58, was admitted to the Queen Elizabeth Hospital in February 1966. Two months previously she had developed a generalized red, scaly rash. The skin of the palms and soles was thickened and fissured. The rash completely covered the body except for a few small patches, as is typical in generalized PRP. A biopsy from the arm showed hyperkeratosis, a little spotty parakeratosis, and some round-cell infiltrate in the upper dermis, findings consistent with PRP.

During the next 3 months there was moderate improvement with betamethasone in doses up to 12 mg daily. Repeated 6-day courses of methotrexate in daily doses of

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2·5 mg, and betamethasone valerate cream, 0·1%, were of no help. The oral corticosteroid therapy led to a rise of blood pressure to 200/115 mmHg, congestive cardiac failure, and diabetes mellitus.

On July 2nd, 1966 therapy was changed to azathioprine, 100 mg twice daily, with rapid stepwise reduction of the corticosteroid dosage. Marked improvement was seen within one week and, by September 1966, the skin was clear except for a little scaling on the face. Azathioprine was gradually reduced and, in February 1967, was stopped because of persistent nausea. A barium meal at this stage showed a hiatus hernia. There was no immediate relapse on withdrawal of azathioprine, but erythema and scaling of face, chest and arms appeared gradually. Azathioprine was therefore resumed in a dose of 50 mg 3 times daily, and the rash disappeared slowly again. The dose was slowly reduced and finally discontinued in October 1967, with no relapse to date. The lowest W.B.C. 1600/mm^{-3}, was recorded when the patient was taking 200 mg of azathioprine daily. Counts of erythrocytes and platelets were within the normal range throughout.

Case 2.—R.G.N., a man aged 50 when treatment was begun in October 1966, was first seen at the Repatriation General Hospital, Daw Park, in 1958. His rash began in 1945 on the face and gradually spread to become almost universal. A diagnosis of PRP was made in 1956. Vitamin A, in doses of 400,000 units daily, helped a little, and betathasmeone valerate cream produced some lessening of the erythema. The skin biopsy was consistent with PRP. He was given azathioprine 50 mg twice daily in October 1966. The rash faded considerably within a month and continued to fade for another month, but he was forced to stop taking the drug because of severe lower thoracic and upper abdominal pains, which began immediately after each dose. Barium meal and electrocardiogram were normal, and the pain ceased when the drug was stopped, only to recur on resuming a dose of 25 mg daily. There was no immediate deterioration of the skin after treatment was stopped, but he relapsed gradually over the next 3 months. Methotrexate in a dose of 5 mg daily for 6 days each month has helped a little, but its effect is much less than that of azathioprine.

Case 3.—V.H.K., a man aged 54, was seen at the Queen Elizabeth Hospital in May 1966 with a rash covering the whole body, which rash had begun 2 years previously on the left leg. Local treatment and a course of methotrexate had not helped. A clinical diagnosis of PRP was made on finding small discrete areas of sparing, and a biopsy from the right arm showed the features expected in PRP. Treatment with azathioprine, 50 mg twice daily, was begun on November 1st, 1966, with slight improvement, which accelerated when the dose was increased to 50 mg 3 times daily in January 1967. Over the next few months the rash gradually cleared completely. In April 1968 he had treatment for coincident solar keratoses and skin cancers. The dose of azathioprine was reduced and stopped in December 1968, with no recurrence to date. No toxic effects were seen at any time.

Case 4.—J.P., a man aged 47, was first seen at the Queen Elizabeth Hospital in July 1967. In his work as a chromium plater he had sustained " chrome burns " to the scalp in May and June 1967, which did not heal but became red and scaly. Later, red blotches appeared on the trunk and limbs, and the whole skin soon became involved. The palms and soles were greatly thickened and fissured. He had a past history of perforated duodenal ulcer. Treatment during the first 3 months with betamethasone 0·75 mg daily, long-acting ACTH and various local applications, was of no help. Azathioprine 50 mg 3 times daily was begun in October 1967 with slow improvement, which was detected by the patient before it could be seen. The skin cleared completely in the next year. The dose of azathioprine was reduced during 1969, but the first signs of relapse occurred when a daily dose of 50 mg was reached. The skin cleared again on 100 mg daily. At present he takes 50 mg daily and gets occasional small red scaly spots which do not trouble him. If he takes a smaller dose, these red spots are more frequent and troublesome and develop into an indurated area which goes down over a period of a week, leaving atrophic spots 2 or 3 mm in diameter. Blood picture, renal function and liver function were checked routinely, but no toxic effects were seen, and there was no exacerbation of his duodenal ulcer.

Case 5.—R.C., a man aged 67, was first seen in June 1968. A rash began in the groin about 30 years previously and spread over the whole body during the next 2 to 3 years. No treatment had been of any benefit. When first seen he had a generalized red, scaly rash, with some small discrete spared areas. Typical follicular plugging was seen on the back of the fingers, and a biopsy showed all features consistent with PRP.

He began taking azathioprine, 50 mg 3 times daily, on July 3rd, 1968 and, within a week, there was some reduction in erythema. The dose was reduced to 50 mg twice daily on July 12th, 1968 on account of abdominal discomfort. There was no recurrence of abdominal pain, and by the end of September 1968, the skin was clear, except for a faint erythema on the trunk and legs. He stopped the drug and was not seen again until May 25th, 1969, when the skin was completely clear. The rash began to recur in February 1970, with patchy erythema and scaling, much less severe than when first seen. One month after re-introduction of azathioprine, 50 mg twice daily, it was possible to reduce the dose to 50 mg daily. Improvement continues. No side effects have been seen.

DISCUSSION

We first tried azathioprine for PRP, without adequate scientific basis, in a case where other measures had failed. The improvement was dramatic. The use of corticosteroids before and at the beginning of therapy with azathioprine may have accelerated the improvement. The prognosis in this and in the fourth patient was very grave before the drug was used. In the first patient the outlook was serious because of the complications of the corticosteroids, the only therapy which had favourably influenced the disease. In the second patient the disease was even more severe, making the chance of a useful existence remote and even his survival doubtful.

The improvement in the other three cases, although slower, was no less impressive. Four of the 5 cases cleared completely, and we are confident that the fifth would also have cleared had he been able to tolerate the drug, which was discontinued because of the acute gastric intolerance, a symptom which is extremely unpleasant, but not apparently serious. The only significant toxic effect seen in the other 4 cases was a transient neutropenia, which responded to a reduction in the dosage.

Experience with the fourth patient has demonstrated unequivocally that improvement was due to azathioprine. Reduction of the dosage has allowed the disease to emerge in a modified form. A slight increase in the dosage is followed in a few days by an amelioration of the rash and a prevention of further outbreaks.

Azathioprine is extensively used in clinical medicine for inhibition of immunological responses. It is a derivative of 6-mercaptopurine, in which an imidazole ring replaces the hydrogen of the SH group. The fundamental mode of action of the drug is not yet understood. It affects purine and amino-acid metabolism in lymphocytes directly, but such effects are seen only at rather high concentrations *in vitro* (Smith and Forbes, 1970). There is evidence that it inhibits the supply of purines by the liver which are required for DNA synthesis in proliferating lymphoid tissues (Chalmers *et al.*, 1971).

Side effects are neutropenia, anaemia (and occasionally thrombocytopenia) and a paralysis of immunological defences against infection. It may also cause biliary stasis which is manifested clinically as jaundice. A few patients cannot tolerate the drug because of acute epigastric pain shortly after taking it. In our experience of this and other patients, such intolerance is never reversible and cannot be overcome by temporary withdrawal of the drug or by the use of very small quantities. The barium meal has been negative in such cases observed by us. The other complications are reversible and the drug can be used again after an interval which has allowed recovery to take place.

It is interesting that the dosage of azathioprine which is effective for control of this disease does not depress immunological mechanisms to the point where these patients become susceptible to the ubiquitous pathogenic micro-organisms. Intercurrent infections have not been a problem. One subject (Case 4) reacted by delayed hypersensitivity to a streptococcal streptokinase–streptodornase antigen, and made a secondary antibody response after challenge with tetanus toxoid, while his skin lesion was satisfactorily controlled with azathioprine (Forbes, unpublished data). We do not claim, therefore, from our evidence, that azathioprine is beneficial in PRP through an immunosuppressive action, or that its efficacy in PRP is good evidence that there is an autoimmune pathogenesis in this disease.

It is our opinion that these cases show that azathioprine is likely to prove a useful drug in cases of PRP, in doses which are well tolerated. It is the only drug which in our experience has produced complete clearing of PRP, although improvement may be slow, and complete remission may take many months. Premature withdrawal of the drug leads to relapse, and small doses may be needed for a long time, but 2 of our cases have now remained clear without treatment for 3 and 2 years respectively.

We thank the Chairman of the Repatriation Commission for permission to include Case 2. Immunological function tests performed on Case 4 were part of research supported by the National Health and Medical Research Council.

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Case Reports

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A BENIGN "MALIGNANT" ULCER

P. C. H. NEWBOLD* AND R. B. STOUGHTON†

Summary.—A case is presented which is not amenable to exact diagno In the presence of active auto-immune thyroiditis, this lady developed a flc phagedaenic ulcer without obvious aetiology. No pathogen could be isola and the lesion does not fit into any neoplastic or pseudo-neoplastic catego Her excellent response to conservative treatment favours a non-malign background, but even long term follow-up will not resolve all doubts, as has received a course of radiotherapy.

CASE REPORT

History

Mrs H. S., a white woman aged 65, was admitted to this clinic on December 17th, 1 She was vague about details, but is adamant that she had never previously had any s trouble, and she could not recall any injury or other predisposing cause. After work in her garden in September 1969, she noticed 3 small red spots like insect bites. Th slowly enlarged and then discharged like ripe furuncles, but did not heal. She was f seen by a dermatologist in February 1970, when she had a single large granulomat plaque on the exterior aspect of the right forearm. There were also a number of sub taneous nodules, some of which felt cystic, arranged in a roughly linear fashion. diagnosis was established, and treatment with local and systemic antiseptics and a fungal agents did not help. For a time the ulcer scabbed over, but, as it enlarged began to weep and to discharge much evil-smelling pus. Pain was slight, and she never had bleeding. Direct questions indicated that she had had no contact wit similar case; she did not have an aquarium or handle fish, nor had she been exposed tuberculosis. Her general health was excellent, and she had never left the Uni States.

Examination

She was a pleasant but extremely slow and apathetic lady, with a husky voice, a pale yellowish-brown skin. The scalp hair was diffusely thin so that she wore a w There were prominent pouches of loose skin below both eyes, with marked reduction facial expression and eye movements. All the deep reflexes were reduced, with p longed relaxation phase. Her blood pressure was 120/70, the pulse was 68/min a apart from her right arm, examination was otherwise within normal limits.

There was a single, irregular ulcer involving most of the radial and anterior aspe of the right forearm (Fig. 1, 2). The edges were solid, elevated, serpiginous, shar defined, and dusky purplish in hue, with no undermining. The surface of the ul

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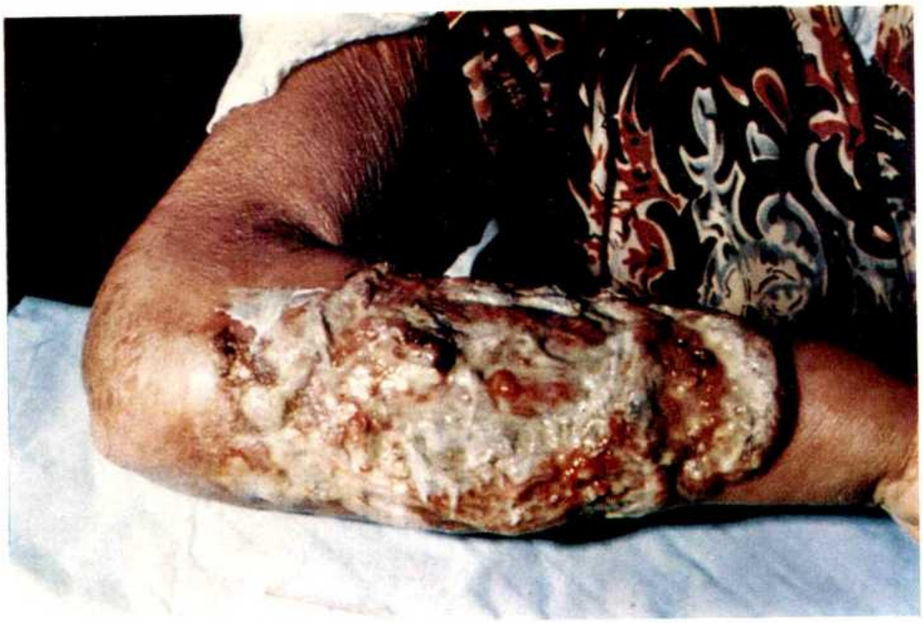

FIG. 1.—Anterior aspect of right arm of patient, showing extent of ulcer.

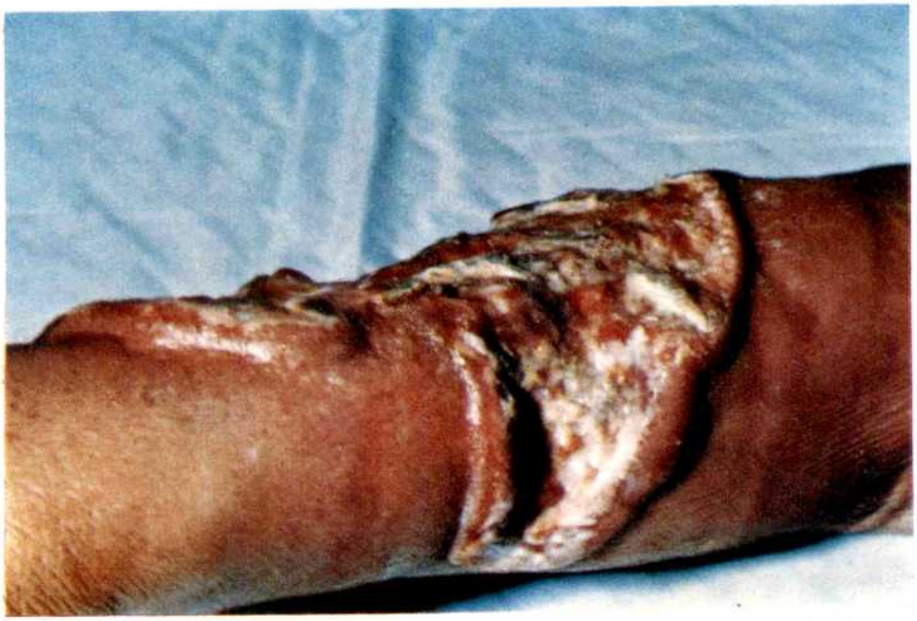

FIG. 2.—Medial and superior aspects of right forearm, showing the rolled edges of the lesion.

was largely covered with purulent debris, but necrosis could be seen in places to include tendons and muscles, and to extend down to the bones, although these were not involved. There were 2 small, non-tender, enlarged lymph glands in the right axilla. Otherwise, apart from some flexion contracture of the right elbow, the limb was normal.

Investigations

Biopsies.—Multiple samples were taken from the edge and from the centre of the ulcer. These showed liquefaction of collagen, with non-specific inflammatory infiltrates consisting of lymphocytes, histiocytes and occasional eosinophils and plasma cells. Some vasculitis was associated, but not the severe necrotizing type. Special stains for fungus and acid-fast organisms were negative. The surrounding skin showed hyperkeratotic epithelium, with some polymorphonuclear leucocytes.

One area in a biopsy suggested neoplastic epithelium, with a high mitotic rate, but a section fixed in Bouin's solution showed that these cells were the mononuclear infiltrate.

Blood bromide level—less than 10 mg/100 ml. This is not significant. Haemoglobin—10·4 g%. Haematocrit 31%. Leucocytes—8761/mm^3—70% polymorphs, 2% eosinophils, 20% lymphocytes, 6% monocytes, and one normoblast per 200 white blood cells. Wassermann test and antinuclear antibody were both negative. Serum proteins and electrophoresis essentially normal. Chest x-ray, and upper and lower GI series were normal. X-ray of right arm showed no bone changes (Fig. 3).

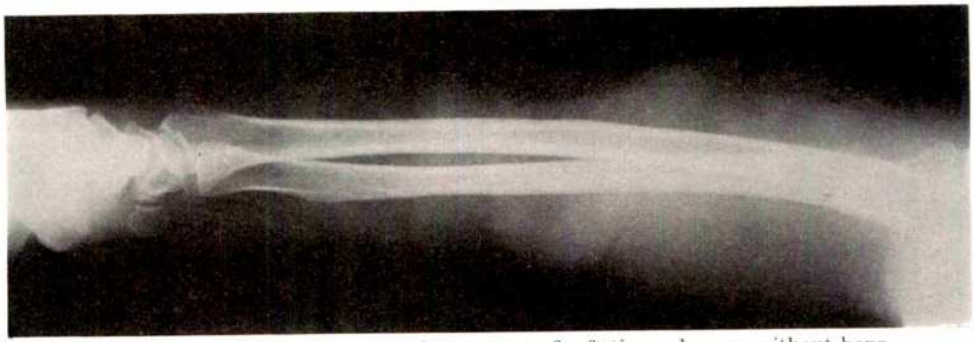

FIG. 3.—X-ray of right arm to show extent of soft tissue damage without bone involvement.

Cultures.—*Proteus mirabilis*, *Pseudomonas pyocyanea*, moderate numbers of mannitol-positive *Staph. aureus*, no fungi and no acid-fast bacilli.

Skin tests.—Negative to PPD, and histoplasmin, coccidioidin and blastomycin antigens.

Thyroid function tests.—Summarized in Table I.

The diagnoses, therefore, were of hypothyroidism and chronic ulcer of uncertain aetiology.

TABLE I

Test	Result
T^3 resin uptake	18·7% (normal: 25–35%)
T^4 (thyroxine)	Less than 1·0 μ I_2% (normal: 2·6–7·1 μg)
Total serum I^2	1·2 μ g%(normal: PB1 + $\ngtr$ 3)
^{131}I uptake	2·1% at 24 hours (normal: 10–40%)
Cholesterol	368 mg% (normal: up to 260 mg)
Cardiogram	Low voltage, QT 0·38, rate 60/min, T wave depression
Antibodies	Anti-thyroglobulin antibody 4 + Anti-thyroid epithelial antibody 3 + Haemagglutinating and precipitating antibody to human thyroglobulin—negative

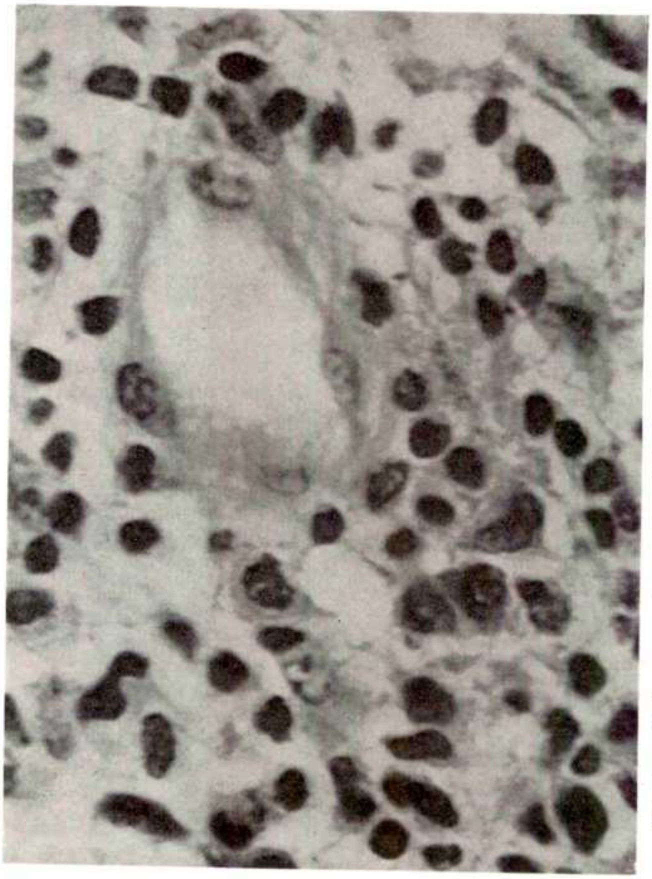

FIG. 5.—High-power micrograph of infiltrate, with giant cells, irregular nuclei, and appearances of malignancy.

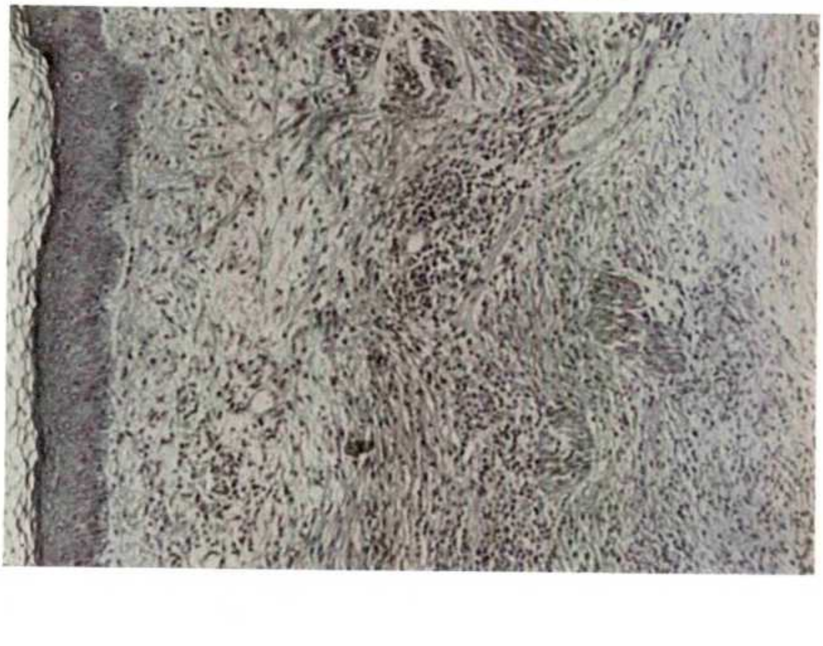

FIG. 4.—Low-power micrograph through edge of lesion, with patchy infiltrate throughout the dermis.

Progress

Treatment consisted of twice daily soaks in aqueous cetrimide, and topical gentamycin cream. She was also started on levothyroxine 0·025 mg/day, and zinc sulphate 220 mg t.i.d., a.c. The thyroid replacement was slowly increased and, in January prednisone 25 mg/day was added to the regime. She had an extensive surgical debridement, and thereafter the lesion steadily improved.

However, samples of the tissue removed showed an infiltrate of large mononuclear cells with irregular nuclei (Figs. 4, 5). Occasional multinucleated foreign body giant cells were seen in the base of the ulcer; the cellular infiltrate extended through fascia and muscle, and was more pleomorphic in the deeper sections. The slides were seen by many pathologists, and the suggested diagnoses included mixed malignant lymphoma, reticulum cell sarcoma, secondary carcinoma, and Wegener's granulomatosis.

The patient returned home for personal reasons, and later had 15 cobalt treatments, a total of about 4000 rad, to the right forearm at a local hospital. Subsequently she had multiple pinch grafts which all took well, and the lesion dried out and healed up satisfactorily. She was seen at her own home on 2 occasions, but would not attend for clinical evaluation, and she has now left the area. Before this, functional and cosmetic recovery were excellent, and there was no sign of metastatic disease.

DISCUSSION

Our initial diagnosis of the ulcerative lesion in this case was pyoderma gangrenosum, which is known even in the absence of colon disease. There was ample evidence for active auto-immune thyroiditis, although this is not a recognized association with pyoderma. It was for the thyroiditis that prednisone was administered, although the favourable response suggested also the possibility that we were dealing with a case analogous to the " malignant pyoderma " of Perry (1968). As the patient's thyroid function improved, she was able to extend the history by adding that her mother and one brother and one sister had similar thyroid disease and facies, while 4 other siblings were normal. Her own appearance and voice had been unchanged since late childhood. However, she could not say definitely when or how her ulcer had started, although the natural history was at least 18 months. It is most unlikely that a lesion of such apparently malignant histology could be present for such a period without metastasis or general illness. However, a study of the literature revealed that the upper limb is a relatively frequent site for sarcoma, that precise diagnosis is extremely difficult and that prognosis is variable and not universally bad (Hare and Cerny, 1963; Atkinson *et al.*, 1963). It is also recognized that a number of other conditions can simulate sarcoma (Genant *et al.*, 1971), and carcinoma is amongst these (Sherwin *et al.*, 1963; Fraser and Kinley, 1968). Another form of pseudosarcoma occurs with fasciitis and myositis (Stout, 1961a, b; Price *et al.*, 1961; Kwittken and Branche, 1969) These odd lesions are especially frequent in the sixth and seventh decades, and commonly involve the upper limb (Stout, 1961a). The aetiology is obscure, but the subcutaneous fat and fascia are involved, and the histological diagnosis offered is commonly fibrosarcoma or liposarcoma. Ulceration is not a feature of these lesions, but their behaviour is benign, apart from a minor tendency for local recurrences (Price *et al.*, 1961). On the other hand, if we accept the diagnosis of pyoderma gangrenosum in the presence of auto-immune thyroiditis, we might expect an abnormal lymphoreticular response analogous to that which has been described in other tissues (Artinian *et al.*, 1971).

If the ulcer has been present longer than we believed, it could be argued that this could itself be pre-malignant (Kanaar and Oort, 1969). It is also tempting to suggest that her combined endocrine and immune disorders could also predispose towards lymphoreticular malignancy (Bierman, 1968). Although the treatments she has had mean that the whole truth can never be known, it remains our strong impression that this lady was suffering from an as yet undefined benign entiry. Her recovery followed radiation therapy as well as treatment with thyroid and corticosteroids. If it had followed the hormones alone, we would have had a stronger case for suggesting a new pseudo-malignancy comparable to lymphomatoid papulosis. This striking entity is histologically terrifying, and yet it clears up spontaneously (Macaulay, 1968). So we offer this case as a contribution to the curious borderline between normality, pre-neoplasia, pseudo-malignancy and neoplasia.

Acknowledgements to Dr Vanderlaan, Dr Abrahamson, Dr Weigle and to other colleagues at S.C.R.F., and to Mr G. Sandford, who took the photographs. We thank Dr Paul Hirsch of Los Angeles for his cooperation in the study of this patient. We gratefully acknowledge the kindness of Westwood Pharmaceuticals Inc. of Buffalo, who defrayed the cost of the colour plates. This work was supported by NIH Research Grant AM 11649.

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UNILATERAL SPIDER NAEVI

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Summary.—A patient with unilateral spider naevi occurring during her second pregnancy is described. Possible mechanisms are discussed.

SPIDER naevi are usually distributed symmetrically in the upper half of the body (Bean, 1958), but there have been 5 previous reports (Pautrier and Ullmo, 1931; Billings, 1963; Selmanowitz, 1970; Aran and Solomon, 1970; Koopmans-van Dorp, 1971) of unilateral spider naevi. We report another case of this unusual condition, which was so extensive that the affected arm was markedly different in colour from the opposite limb.

CASE HISTORY

A woman aged 24 developed many spider naevi on the entire left upper limb at the third month of her second (a twin) pregnancy. These extensive spider naevi had not occurred during her first pregnancy 3 years earlier. The spider naevi, which were classical in their appearance and present in their thousands, increased in size and number until delivery; relatively insignificant naevi were present on the face and right arm.

The patient also had hypertension and increasing albuminuria in the last trimester of the present pregnancy, her blood pressure reaching 170/120 and the albuminuria 10 g/l. General examination revealed no other abnormality of the left arm; in particular, the peripheral pulses were equal and no bruit could be heard over the heart or limbs.

Labour started spontaneously at 34 weeks. All cutaneous lesions had cleared within 5 weeks of delivery, with the exception of a pulsatile lesion on the left thumb. The babies had no spider naevi.

Investigations.—The urine contained 2–10 g/l of protein; blood urea was 26 mg%; creatinine clearance was 76 ml/min. Liver function tests were normal, with the exception of the alkaline phosphatase, which was increased to 43 units, compatible with a toxaemic pregnancy. Twenty-four hour urinary oestrogen levels were high (mean of 8 determinations from 30–34 weeks: 29 mg/24 hours).

We considered the possibility that other localized abnormalities of the left arm might exist. Infra-red photography did not suggest the presence of a deeper vascular abnormality. The possibility of underlying arterial shunts was investigated by assessing venous Po$_2$: no difference was detected (right, 38 mm; left, 41 mm). At the 34th week histamine (0·1 ml of the 1 mg/ml solution) and serotonin (0·1 ml of 10 mg/ml solution) were injected intradermally. The erythema, weal and the time courses were equal in both arms.

Six weeks after pregnancy it was attempted to induce the development of spider naevi by applying oestrogens to the right and left arm; 0·1% and 1·0% concentrations were applied 3 times a day for 4 weeks; no spider naevi developed with either concentration.

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DISCUSSION

Although spider naevi are usually symmetrical, 8 patients with asymmetrical spider naevi have been reported (Pautrier and Ullmo, 1931; Billing, 1963; Selmanowitz, 1970; Aran and Solomon, 1970; Koopmans-van Dorp, 1971), and 7 of these were females. In the male patient the telangiectasia was present at birth, in one female at puberty, and during pregnancy in the other patients. It is clear that the distribution of these spider naevi is of naevoid origin; in the female patients it is presumably dependent on oestrogen stimulus at either puberty or pregnancy. It is of interest in our report and in 2 further cases (Pautrier and Ullmo, 1931; Koopmans-van Dorp, 1971) that the patients had no abnormal naevi in the first pregnancies; the naevi developed during the second pregnancies. It may be that certain of these naevi need priming of the target end organs before they appear, or that the level of hormonal stimulation is critical. Only in the present case were the naevi associated with a twin pregnancy. Our investigations, however, gave no further information on the mechanism of these asymmetrical lesions, which apparently represent a naevoid predisposition of target end organs.

We wish to thank Mrs L. Lane for secretarial assistance.

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Comment

ELECTRON MICROSCOPY: A FUNCTIONAL APPROACH

Like it or not, those of us involved in dermatological electron microscopy in Britain have to admit that the fruits of our endeavours have been only too sparse during the last decade or so. Luckily there have been exceptions, and Professor Breathnach's recent book, *An Atlas of the Ultrastructure of Human Skin*, happily reaffirms that simple and practical problems can be posed in such a way that ultrastructural investigation may help to solve them. Nevertheless it is still fairly obvious that, during most presentations involving electron microscopy, the art lovers and snoozers far outnumber those with even a reasonable understanding of the topic; and who can blame them? Unfortunately, in few fields of research is segregation between enthusiast and non-enthusiast more complete. It does not take long after a real commitment to the discipline to realize that the language you are learning is indeed foreign to most. This comment is intended primarily for those who find themselves perpetually perplexed by the increasing number of papers involving electron microscopy.

Although photographs never tell lies, the interpretation of them, particularly if it is not appreciated how they were obtained, may lead frequently to a conclusion far from the truth. At the risk of appearing too cynical, it should be pointed out that published pictures and slides at meetings are the pick of perhaps hundreds of micrographs, and their choice is inevitably subjective and biased. It is also worth assuring oneself, before swallowing any conclusions hook, line and sinker, that the micrographs are likely to be representative of the general state of affairs, and are not based on evidence that is only too thin (50 nm) and taken perhaps from a solitary cell. How do we know which and what not to believe? The technical quality of micrographs is often easy to discern. Suspicion is aroused by unacceptable (poor) cellular preservation. Excessive artifact and poor focusing are further signs of faulty technique. Perhaps most aggravating of all is the tendency of some journals to shrink high quality micrographs into such miserably small replicas that interpretation by even the keenest eyes becomes impossible. Good micrographs should reveal their point clearly; and, if an author cannot persuade an observant reader that *a* is like *b* and that both differ from *c*, he should not be believed on the basis that " he knows better than I ". Confirmation of results by other workers is essential in electron microscopy, more so than in most other fields; and, although a new observation should certainly register, similar evidence should be sought before accepting anything as gospel. To sum up: the limitations of the technique, the subjective nature of selection and the technical quality of the procedures involved are all points to be considered when assessing the merits of any paper. Providing that attention is paid to these, standard transmission electron microscopy can further functional understanding when used by itself, with simultaneous cytochemical procedures, with radioactive tracers and in combination with other subcellular techniques. Random examples of work involving each of these categories show what varied contributions they have already made.

A frequent and often justifiable criticism of standard electron microscopy is that its static and structural nature can be positively misleading when trying to unravel a dynamic and functional problem. It could be argued that past research involving pemphigus, mycosis fungoides and skin-stripping provide examples. There was a good reason why the epidermal intercellular cement substance came under scrutiny (Hashimoto and Lever, 1967, 1970) even although previous electron microscopic evidence had suggested that alterations in the tonofilament–desmosomal complex were primarily involved in the acantholytic process (Wilgram *et al.*, 1961; Braun-Falco and Vogell, 1965). The reason had nothing to do with electron microscopy but was simply the fact that Beutner and Jordan (1964), using fluorescent microscopic techniques, had described the presence of an antibody reacting with the constituents of the intercellular space. In 1968 Lutzner and Jordan described the ultrastructural features of the characteristic cell seen in the Sézary syndrome (Sézary, 1949). The nucleus was strikingly irregular, indented, lobulated and serpentine in outline. They noticed fewer but similar cells in the infiltrate of mycosis fungoides, but found none in normal blood, blood from patients with chronic lymphatic leukaemia, a lymph node from a patient with Hodgkin's disease and skin from a variety of benign and neoplastic cutaneous lesions. A characteristic cellular marker was obviously an attractive hope, although Lutzner *et al.* (1971), in a later publication, began to query the specific nature of it, as they had now noted such cells in a case of reticulum cell sarcoma as well as in 2 cases of lichen planus. More recently Flaxman *et al.* (1971) have detected similar cells in a variety of randomly selected non-lymphomatous dermatoses and therefore consider them to be non-specific. Ultrastructural changes occurring as a result of cellophane tape stripping of the horny layer of human epidermis (Pinkus, 1951) have been investigated by Mishima and Pinkus (1968), Mishima (1966), Mottaz and Zelickson (1970) and Mottaz *et al.* (1971). In spite of similarity in their methods (although different sites were studied), the changes which caught their eyes were clearly different. Mishima and Pinkus were impressed by the dissolution of the tonofilament–desmosomal connections, keratinocyte phagocytosis of retracted desmosomes and the presence of melanosomes within phagocytic vacuoles of Langerhans cells (Mishima, 1966). Mottaz and Zelickson were struck initially by the phagocytic nature of the keratinocytes which contained phagocytosed parts of Langerhans cells, and later by the fact that many melanocytes contained melanosomes within autophagic vacuoles, showed increased tyrosinase activity and phagocytosis of parts of Langerhans cells. The pictures presented in the publications are all convincing, and comparison of their interpretations of the overall changes serves as a reminder that different observers note different things when faced with similar material. In spite of such apparent about-turns and differences in interpretation, electron micrographs occasionally reveal structural appearances which positively demand a functional explanation. Distinctive granules in Langerhans cells (Birbeck *et al.*, 1961; Sagebiel and Reed, 1968), Langerhans cells in the epithelia of the gingiva (Schroeder and Theilade, 1966) and cervix (Hackemann *et al.*, 1968), in dermis (Zelickson, 1965), stroma of benign appendage tumours (Hashimoto and Tarnowski, 1968), hyperplastic lymph nodes (Jimbow *et al.*, 1969; Shamoto *et al.*, 1971) and in the lesions of histiocytosis X (Basset *et al.*, 1965) are all entirely structural observations which must have important implications for the function of this mysterious cell. There will always be a place for such talented observations, although not all are so fortuitous as they might seem.

Electron microscopic cytochemistry is still in its infancy. The limitations are similar to those of conventional light histochemistry, although the techniques are frequently even more capricious. Two broad divisions of this approach separate techniques involving the addition of a chemical which reacts with or digests tissue constituents from those which deal with the subcellular detection of enzyme activity. In the first are the commonly used fixatives, such as osmium tetroxide and glutaraldehyde, as well as such routine stains as uranyl acetate and lead citrate, which increase the differential electron scattering power of various components of tissues and cells. More specific dermatological applications include the use of osmium iodide to stain membrane coating granules (Breathnach and Wyllie, 1966), osmium zinc iodide to render parts of Langerhans cells and granules preferentially visible (Niebauer *et al.*, 1969), ruthenium red to stain the cell surface coats of keratinocytes in pemphigus vulgaris (Hashimoto and Lever, 1970), lanthanum nitrate to outline the cell surface coat of the Langerhans cell and to permeate the intercellular spaces (Hashimoto, 1970) and differential enzymatic digestion to determine the nature of cytoplasmic components of keratinocytes (Wolff and Schreiner, 1971). Specific antibody can be bound covalently to ferritin (Singer, 1959) or enzymes such as horseradish peroxidase (Nakane and Pierce, 1967) and used to detect the site of antigen at a subcellular level. Ferritin is a crystalline protein which is unique in that it contains a considerable amount of iron in ferric hydroxide-phosphate miscelles. It is electron dense but, owing to its large molecular size (460,000), ferritin labelled antibody penetrates tissue poorly and may not react with enough intercellular antigen to provide sufficient contrast at the sites of antigen–antibody reactions. These problems are less evident using horseradish peroxidase, which has a smaller molecular weight; but, once inside the cell, it has to be detected by a further cytochemical reaction (see below) which results in the deposition of an electron dense reaction product.

Techniques involving subcellular detection of sites of enzyme activity were put on a firmer basis when Sabatini *et al.* (1963) recorded that certain dialdehydes (particularly glutaraldehyde) not only fixed and maintained cellular structure but also preserved enzyme activity. The tissue is incubated with a specific substrate and, if the reaction product is not already electron dense, it is captured by the addition of a further substance to the incubating medium. In Barka and Anderson's (1962) modification of Gomori's (1952) lead sulphide method for the detection of acid phosphatase, sodium β-glycerophosphate is used as a substrate, and the phosphate released is captured by the addition of lead nitrate to the medium. If all goes well, insoluble electron dense lead phosphate is precipitated at the site of enzyme activity. This technique is becoming more frequently used, as membrane-bound acid phosphatase activity is now accepted as a marker of lysosomes (Novikoff, 1963). Horseradish peroxidase can be detected by its reaction with the substrate H_2O_2; the oxygen released is captured by 3,3′-diaminobenzidine, which is oxidized to form a non-crystalline insoluble electron opaque product after post fixation with osmium tetroxide (Karnovsky, 1965). Different workers (Novikoff *et al.*, 1968; Hunter *et al.*, 1970; Maul and Romsdahl, 1970) arrived at similar conclusions regarding the site of tyrosinase activity within normal and neoplastic melanocytes. Detection of that enzyme is particularly convenient, as the product of its interaction with substrate (tyrosine or DOPA) is the electron dense melanin.

Electron microscopic autoradiography has extended the possibilities for experimentation and further levels of analysis. Jacob (1971), in a recent comprehensive review, pointed out that the resolution is about 10 times better than with conventional light microscopic autoradiography and that the limiting factors are autoradiographic rather than optical. Although many of the procedures are time-consuming and tricky, Nakai and Shubik (1964), Blois and Kallman (1964) and Zelickson *et al.* (1965) have, for example, already revealed the scope of the technique when using labelled DOPA to investigate the intracellular site of melanin synthesis.

Standard electron microscopy, with or without combined cytochemistry and autoradiography, can therefore be a most important tool for understanding cellular function. Some might argue, however, that most benefit is derived when it is used as an integral part of a much broader research programme. The classic experiments of Seiji *et al.* (1961), which used enzymic estimations and electron microscopic monitoring of pigment cell fractions separated by density gradient centrifugation, finally led to acceptance of the idea that premelanosomes, not mitochondria, were the site of melanin formation. Everybody is waiting with bated breath to see who will be the first to carry out similar work on the Langerhans cell granule. Unfortunately, a source containing a suitable concentration of this cell is not yet readily available.

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A REVIEW OF THE RECENT LITERATURE ON DISEASES OF THE VULVA—PART II

VULVITIS: INFECTIONS

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VULVITIS

THERE have been several suggestions in correspondence to journals (Tann, 1968; Breen, 1971; Stallworthy, 1971) that the wearing of tights may cause vulval irritation, Bull (1969) suggesting that the symptoms are more likely to be related to the Pill; Kaye (1970) suggests that vaginal deodorant sprays may irritate. No controlled study seems to have been attempted.

Davis (1971) describes what he believes to be an entity, salivary vulvitis as a consequence of sexual exposure. He has found 6 cases with non-specific appearances but all responding promptly to avoidance of the contact. Meehan *et al.* (1969) note another rare cause of vulvitis—secretion of mucus from vaginal adenosis, which in severe cases can lead to maceration of the labia majora and even to the suspicion of incontinence.

Lipschutz's ulcer of the vulva is often regarded as a rather unsatisfactory diagnosis comprising several different entities (Rook *et al.*, 1968; Gardner and Kaufman, 1969). Thiers *et al.* (1969) report a case with undetermined aetiology in a woman aged 24. However, Boyce and Valpey (1971), in reporting several cases of acute ulcerative vulvitis, wonder if the name could be applied here; the ulcers were like those described in the shallow type of Lipschutz ulcer. They feel that otherwise their cases are in a category hitherto undescribed. The cases all occurred in wives reunited with servicemen returning from Asia. Symptoms developed 12–48 hours after coitus, and painful shallow ulcers were seen on the opposing surfaces of the labia minora. No evidence could be found of a specific viral or bacterial origin, and the husbands were unaffected. The ulcers seemed to clear rapidly with symptomatic measures.

INFECTIONS

De Costa (1969) gives a comprehensive general account of infections of the vulva and vagina. He emphasizes that more than one cause may operate in a given patient, and that the role of the contraceptive pill in *Candida* infection is not yet clear. Heller *et al.* (1969) analysed 50 cases of vulvo-vaginitis in children and found a specific aetiology in about two-thirds; these included cases from viral, bacterial and parasitic infections, 2 from foreign bodies (one an earth worm) and no fewer than 8 with " toxic-allergic " vulvitis and perineal rash suspected to result from a bubble bath of unstated composition. One wonders if this latter is the female counterpart of the scrotal rash described in relation to Sterzac by Baker (1969). The authors made a painstaking check on bacteria from vulva, vagina, rectum and the urine; similar tests on normal control children also yielded many species of bacteria. In practice they find that, if there is purulent vaginitis for no apparent cause, it will respond to penicillin and that the non-purulent type responds to general hygienic measures. They, too, stress the probable multi-factorial aetiology.

Josey *et al.* (1969) have written a useful comprehensive review of virus and virus-like infections of the female genital tract. Nicol (1971) briefly in 2 articles without references gives a clear and deliberately didactic account of sexually transmitted diseases apart from syphilis and gonorrhoea. There is a particularly valuable, succinct account of the present status of chlamydiae and mycoplasmas in genital infections.

Stewart (1968) reviews ulcerative and hypertrophic lesions of the vulva in the tropics; they may be related to tuberculosis, filariasis, schistosomiasis, granuloma inguinale and lymphogranuloma inguinale (lymphogranuloma venereum).

Chlamydiae

In 1897 Neisser recognized a non-gonococcal form of conjunctivitis in the new-born, and its possible source in the birth canal of the mother. Later, inclusion bodies were found in the conjunctivae of such babies, similar to those in patients with trachoma (hence the term TRIC—trachoma/inclusion conjunctivitis), and also to those in non-specific urethritis (NSU) and in the genito-urinary tracts of the parent. It is now possible to grow the agent in eggs and to study it by other methods. These researches formed the subject of papers by Jones *et al.*, Al-Hussaini *et al.* and Dunlop *et al.* (1964), dealing with its isolation from the urethra, cervix and eye, its cytology in culture, and the clinical findings. It was possible to conclude cautiously that the TRIC agent was involved in some genital infections. The relevance of this work to the dermatologist lies not only, and not so much, in the question of this organism in relation to NSU as in its suspected relation to lymphogranuloma venereum (LGV).

Wang and Grayston (1970), with an immunofluorescence test, claim to have found distinct types of chlamydiae associated with LGV. Other workers, however, have found that the strains associated with LGV differ from the TRIC strains only in pathogenicity in experimental animals, and that sometimes only knowledge of the clinical source determines its classification as a TRIC or LGV strain. Philip *et al.* (1971) for this reason embarked on a study of chlamydiae in patients with LGV, urethritis (NSU or post-gonococcal), syphilis and gonorrhoea in an area (District of Columbia) where the conditions are prevalent. In all, 155 subjects (patients or their sexual contacts) were studied, using a variety of investigative methods. Although Philip *et al.* acknowledge the fact that they had no control series, they feel that the association of chlamydiae with urethritis has yet to be proved to be constant and of aetiological importance; as regards LGV, the association is fairly convincing, but while the strains isolated in LGV proved to be of expectedly high pathogenicity, and those isolated from urethritis cases were of lower pathogenicity, the question of their specificity has not yet been finally decided.

Kerk and Schilling (1969) made a study of lymphographic findings in 19 patients with various venereal diseases. They found that filling defects in inguinal glands in 5 of 12 with lymphogranuloma inguinale (i.e. LGV) were striking and could mimic those caused by metastases.

Mycoplasmas

The nature, neither bacterial, viral, fungal, chlamydial nor Rickettsial, of these organisms and their status in disease is summarized in the *Lancet* (1970). Their presence in many genital conditions is not denied, but their causal significance remains to be decided. Since they can be found also in 50% of sexually active women, and to a less extent in other groups, it may be that they are sexually transmitted but not pathogenic. Russell and Fallon (1970) assert that they can be found in blood cultures following dilatation and curettage and in Bartholin's abscess, amongst other conditions, and are inclined to accept them as pathogens. Jones (1970) is more sceptical, but suggests that their role in vaginal discharge should be studied, as it easily could be, by routine culture for mycoplasmas.

Viruses—Miscellaneous

Accidental vaccinia with vulval involvement, a case with reference to previous examples, is reported by Hutfield (1968a); both the cervix and the skin were involved, raising suspicion of a herpetic infection, but vaccinia virus was isolated. A further proven case is described by Andreev *et al.* (1969).

James (1968) describes a case of what was diagnosed clinically as vulval orf in a child living on a farm.

Gardner and Kaufman (1969) say that the vulva is a site of predilection for mollusca contagiosa; British dermatologists until recently would have said that the lesions were most often found on the trunk in children. However, Cobbold and MacDonald (1970) compare the incidence of mollusca in the skin department and the VD department of a London hospital. In the whole of 1967, 14 new cases, on the trunk and limbs, were seen in the skin department whereas in 3 months of 1968, 23 new cases, all in the genital area, were seen in the VD department. These authors review previous papers, mostly arising from armed forces material, suggesting the sexual transmission of this condition, and assume that a long incubation period may have made contact-tracing a failure. However, Mandel and Lewis (1970) describe mollusca contagiosa, confirmed by light and electron microscopy, on the thigh of a child of one year, noticed at the age of one week; and postulate either a shorter incubation period, or infection *in utero* via the vagina—although no lesions were known to be present in the parents.

Warts

Although vulval warts are rare before puberty, they may be encountered in children, as is agreed in the texts mentioned previously; Grace *et al.* (1967) report 3 cases and stress that they may easily be misdiagnosed, particularly if solitary.

As regards treatment, the counterpart in women of the work of Halverstedt and Parry (1969) does not appear to be recorded; these authors found instillation of thiotepa into the urethra of a man with multiple lesions effective. Ostergard and Townsend (1969) describe successful treatment with cryosurgery in 17 patients, although they do not state whether they tried it unsuccessfully in others. The method utilizes a probe cooled by liquid nitrogen to a temperature of −160°C. Ostergard and Townsend carried out this treatment without an anaesthetic (2 cases), under general anaesthesia given for delivery (2 cases) and under local anaesthesia in the remainder. They say that, for success, the frozen area must extend through 1–2 mm of normal skin round the lesions. Treatment in instalments is given for massive infections. Healing occurs in 4–6 weeks, following pain, oedema, local gangrene and sloughing.

Electron microscopy has perhaps more to offer in this field than in many others, since it provides diagnostic criteria for warty lesions, so that relationships between genital warts and warts elsewhere may be established. Oriel and Almeida (1970) demonstrated virus particles in genital warts, and the particles looked similar to those found in warts elsewhere, although there were some differences in antigenic structure (Almeida *et al.*, 1969; cited by Almeida and Oriel, 1970 and Oriel, 1971a). The size of the intranuclear particles (about 55 nm diameter) puts them in the papilloma virus group, and these particles correspond to the inclusions seen by light microscopy.

Oriel (1971a) has discussed the recorded and natural history of genital warts in detail. Warts gradually emerged as an entity apart from syphilis and gonorrhoea, and then were thought to be caused by dirt or discharge before their similarity to skin warts was recognized at the end of the 19th century. The nature of the relationship, however, is still in doubt. Penile warts inoculated elsewhere sometimes resulted in warts at the injected site, but the experiments were not conclusive; vulval warts were not used, and the induction of genital warts from skin warts was not attempted. Sexual transmission of genital warts is generally accepted, but there is uncertainty as to whether this is simply an occasional event with a single infecting agent, as seen in scabies, or the usual or only method of transfer, in which case the virus of skin and genital warts may or may not be identical.

Oriel studied 332 patients (191 men, 141 women) with genital warts, excluding those with warts restricted to the anus, over more than 2 years; and bases his findings on the history, examination, course and checking of contacts. As a control group he used 97 men with gonorrhoea, but no warts.

He concludes that, in men, lesions could be divided into hyperplastic and sessile warts and those like ordinary skin warts. In women, a sessile group was not distinguishable; in early cases (duration one month or less) the distribution was mainly at the posterior part of the introitus, followed by the labia; later, more were found on the labia and in the vagina, cervix and perineum. In both sexes there was a striking absence of spread to buttocks and thighs. Pregnancy (13 patients) was associated with much more florid warts, which often disappeared spontaneously after delivery.

He attempted to trace sexual contacts of these patients over an arbitrary 9 months before the warts appeared, and also subsequent to their appearance. Ninety-seven people were found and 62 of them later developed genital warts; not all could be followed up for 9 months; but, on the available evidence, the incubation period appears to range between 3 weeks and 8 months, with an average of 2·8 months. There was an impression that the longer the warts were present, the less infectious they were. One patient produced 3 contacts, all developing vulval warts.

A group of 32 patients with genital warts and only one sexual contact during the preceding 9 months was studied in an attempt to elucidate the relationship between skin and genital warts; the transformation seemed possible only in 2 cases. In the whole study, 4 men and one woman had genital warts which looked like skin warts—in 3 of the men and in the woman, skin warts were present elsewhere; it seems that this may be a small distinct subgroup, and one where incidentally the warts tend to be difficult to treat. The incidence of skin warts in similar racial groups with and without genital warts was compared and found to be almost equal. The remainder of the cases, with the age incidence similar to that of gonorrhoea, appearance of warts at sites of coital injury, and evidence of transmission sexual partners, suggest a sexually transmitted disease without any relationship to skin warts.

Oriel (1971b) has now studied patients of both sexes with warts restricted to the anal area, as well as anal and penile warts in a group of known homosexual male patients. There seemed to be an incontrovertible association of anal warts with anal coitus. The pattern of penile and anal warts in these patients, however, did not support the theory of solely sexual transmission of the virus.

Malignant transformation of warts.—The presence of cervical warts raises the possibility of some relationship to cervical malignancy. The possibility of malignant transformation of external genital warts has long been discussed. Oriel and Whimster (1971) have now added to this subject, at least in the male. They describe the presence of virus particles occurring in perianal warts recurrent at the same site, a few weeks after excision of a similar lesion; histological examination showed an area of *in situ* malignant change as well as the morphology of a wart. The changes were not those of a podophyllin effect. With this new evidence to hand, it is of interest to consider earlier accounts of possible malignant change. Case reports abound, and their titles may mislead. Some deal with malignant transformation of essentially ordinary but florid and persistent lesions. Some are concerned with the giant condyloma acuminatum described as an entity by Buschke and Loewenstein in 1925 (Loewenstein, 1939) thought to be clinically malignant—burrowing, spreading, recurring—but histologically benign; although, as to the latter, opinions have differed.

In the first category comes the report of Embrey (1961), with a well-documented report of the development of a vulval squamous carcinoma with metastases, and the comment of Gardner and Kaufman (1969) that they have met such cases. Čečuk and Prpić (1969) describe florid warts, resistant to treatment, where they resorted to radical surgery and grafting as they feared the development of malignancy. Sonck (1971) gives a very full account of vulval warts ending eventually with a carcinoma and many metastases—again, the lesions seem to have been extensive, but basically ordinary, condylomata acuminata. This patient had had unspecified doses of x-rays for the warts and then to induce the menopause. A further case has been reported by Kerl and Pickel (1971); here, too, radiotherapy (4500 R) had been given before the development of the carcinoma.

As regards the giant condyloma acuminatum, many reports concern males, to whom the lesion, often preputial or perianal, was thought at first to be confined. Such cases are described by Sims and Garb (1951); Machacek and Weakley (1960); Siegel (1962), where a giant wart was repeatedly examined as it extended, burrowed and recurred, and thought ultimately to be histologically malignant; Thivolet *et al.* (1969) and Dawson *et al.* (1965), with 4 patients where frankly malignant changes were found in 2, and *in situ* changes in one; and Becker *et al.* (1969), who advocated prophylactic circumcision, and even amputation of the penis, although attacked on that score by Weakley (1969). Judge (1969), however, described a case at the vulva with extensive spread leading to rectal obstruction, but histologically benign. Evans and Dische (1969) had a patient with what seemed a similar giant vaginal lesion, histologically benign; electron microscopy and culture failed to demonstrate the virus. Foye *et al.* (1969) described 2 cases, under the title of verrucous carcinoma of the vulva, which may be similar.

Davies (1965) attempted to clarify the histology by analysing 100 specimens diagnosed as carcinoma of the penis; among these she found 4 basal cell carcinomata, 5 miscellaneous tumours, and 35 true squamous cell carcinomata. The rest she divides into 24 giant condylomata acuminata, of which one was accepted as showing malignant change, 17 lesions which she calls solid papillary epitheliomata, and 15 malignant condylomata. She believes the giant condyloma is basically not malignant, although extensive, and has a definite histological pattern. The malignant condyloma is histologically intermediate between the giant condyloma and a squamous carcinoma, but has a short history, whereas the giant lesion has a long one. She believes the solid papillary epithelioma is distinct histologically and clinically.

Clearly there is need of a similar histological analysis, preferably with correlated viral studies, of the vulval lesions; size and potential malignancy may vary independently, and presumably much also depends on anatomical conditions and treatment.

Herpesvirus Hominis (*HVH*)

Hutfield (1968b) reviewed fully the historical and clinical features of what was then termed herpes genitalis. Friedrich *et al.* (1969) describe the electron microscopic findings in a case with florid lesions of the vulva and elsewhere, and Dickie (1969) a series of 20 cases with cytological diagnoses, both accounts with many references. Hutfield and Longson (1968) noted the isolation of " an atypical strain " in genital infections, and that inclusion bodies in the cells of the cervical smears must be distinguished from malignant changes. Subsequent work has confirmed that HVH infection may be by distinct types, 1 and 2, and that genital infection is almost always by Type 2. Experimental and epidemiological evidence to this effect is given by Kleger and Prier (1969), and by Josey *et al.* (1968) in a valuable review which deals also with the association of HVH Type 2 infection with cervical carcinoma; much of the virological work was stimulated by the epidemiological evidence linking carcinoma of the cervix with venereal disease, which made HVH a likely candidate as a possible cause.

The venereal transmission of HVH Type 2 infection has been well established; for example, by Nahmias *et al.* (1969b), who investigated contacts of patients with herpetic infections (and stressed that cervical lesions may occur in the absence of vulval signs), and Amstey and Balduzzi (1970b), who found associated venereal disease in 7 of 15 patients with HVH Type 2 infection.

Evidence for the relation of Type 2 infection to carcinoma of the cervix is based mainly on antibody studies in groups of patients with carcinoma, *in situ* and invasive, and without. Studies indicating that the incidence of infection is significantly greater in the carcinoma group have been made by Rawls *et al.* (1970a), Royston and Aurelian (1970), Nahmias *et al.* (1970b), and Plummer and Masterson (1971). The difficulty of finding a control group matched as regards race, social status and age is acknowledged by all the authors, particularly Rawls *et al.* (1971). Rawls *et al.* (1970b) have shown that, in New Zealand, as opposed to the United States, there is no significant difference between control groups and those with carcinoma and carcinoma *in situ*. Centifanto *et al.* (1971) also stress the lack of definite proof of a causal relationship, even where correlation has been shown, and advocate direct cell culture techniques on the cervix as a better method. Plummer and Masterson (1971) discuss the cross reaction between Type 1 and Type 2 antibodies, both of which will to some extent neutralize Type 2 and Type 1 virus respectively. Hence the interpretation of serology can be difficult, and even in part subjective. These authors are not convinced that the differential between Type 2 antibodies in patients with or without cervical carcinoma is as marked as has been alleged, or that the overall rate of infection with Type 2 virus is as common.

Some authors describe dual or intermediate antibodies, implying past infection with both types of virus, and it is likely, as shown by Nahmias *et al.* (1970a), that a new Type 2 infection may occur in a subject who has already had a Type 1 infection; their work deals with the whole pattern of Type 1 and Type 2 antibodies at various ages in various population groups. London *et al.* (1971) have found that monkeys (*Cebus* species) may serve as an experimental model for human Type 2 infections and their sequelae, and have shown that homologous reinfection can be produced by reinoculation, a point upon which studies in humans have hitherto not been definite. Nahmias (1971) points out that the clinical importance of this finding has yet to be assessed.

Amstey and Balduzzi (1970a) have distinguished a different strain in one of 18 patients with Type 2 infection. Jeansson and Molin (1971) suggest a link between genital herpes infection and non-specific urethritis. Rodin *et al.* (1971) have found that the virus can be transported as safely in Stuart's medium as in special virus media.

There is no doubt that infection of the foetus or infant with HVH may occur, and may be serious. Nahmias *et al.* (1968) suggest that, for this reason, children with genital HVH lesions should be watched into adult life. South *et al.* (1969) described congenital malformation of the central nervous system as a probable consequence of maternal genital HVH infection. Naib *et al.* (1970) demonstrated an increased abortion rate in women with evidence of past HVH infection; and Goldman (1970) found herpetic involvement of the endometrium in an abortion. Nahmias *et al.* (1970c) reviewed the subject (140 references), commenting on the possible mechanisms, and describing clinical syndromes in the neonate, diagnosis and treatment.

Amstey (1971) discusses the management of pregnancy complicated by HVH Type 2 infection in 7 patients. In 4, the viral cultures were positive at 8–21 weeks of pregnancy, but negative later, and delivery was normal. In 3, infection developed at 28 weeks or later; in spite of Caesarean section in all cases, one baby died and HVH was found in the liver and adrenals. The infection is usually acquired during delivery, or by ascending infection following prolonged rupture of the membranes. Amstey stresses the value of culture, which can give useful information within 2 days, since a cervical smear showing viral changes cytologically may not mean that there is an active infection there; cytology of vaginal or vulval ulcers may, however, give helpful information within hours. That the occasional genital infection with HVH Type 1 is not exempted from this threat is shown by the case report of Catalano *et al.* (1971), where the infant died.

Tuberculosis

Shah and Shah (1968) report a case where an ulcer of the vulva (non-specific histologically), glands (suggestive of tuberculosis histologically, although no mention is made of acid-fast bacilli), and a sinus in the groin were associated with elephantiasis of the vulva. All the signs settled with anti-tuberculosis treatment. Tuberculosis of the vulva is very rare in all parts of the world, and in the literature reviewed here is said to comprise only 1% of cases of tuberculosis of the lower genital tract.

Lawson and Stewart (1967) and Stewart (1968) discuss its differential diagnosis and point out that it may occasionally cause an isolated infection of Bartholin's gland; or inguinal adenitis, resulting in lymphoedema of the labia without any apparent vulval lesion.

Chancroid

Chancroid is rarely seen here, but Kerber *et al.* (1969) report 90 cases, for the condition is common in the American forces in Vietnam. *Haemophilus ducreyi* was not isolated, and LGV was not excluded by Frei and by complement fixation tests, but Gram-negative rods were seen in all cases where they were sought, and the authors feel the clinical picture was distinctive. Although tetracycline had been thought to be effective alone, it seemed here that sulphonamide, alone or in conjunction with tetracycline, was needed; it is possible that other workers had included cases of other conditions, or that the organism had been made resistant in Vietnam by the unofficial and haphazard taking of antibiotics.

Although commoner in men, chancroid has been reported in a woman by Manuel and Vermeer (1971).

Granuloma Inguinale

This condition too, caused by *Donovania granulomatis*, is not often found in this country, even in immigrants, but is prevalent elsewhere.

Thew *et al.* (1969) reported 4 cases, all in women, and in all of which Donovan bodies were seen in crush preparations. They responded to ampicillin, including one which had recurred following tetracycline.

Davis (1970), however, reported on 14 cases (4 in women), also confirmed on crush preparations, which healed well on tetracycline, 2 of which had failed to improve when given ampicillin (Gardner and Kaufman (1969) recommend tetracycline, Lawson and Stewart (1967) mention also the use of streptomycin). Eight of the 10 men in this series were homosexual; the lesions are often perianal in such men, and an organism like the causative one can be found in the stool; Davis therefore suggested that the gut may be the source, particularly as the disease is rarely found in prostitutes or in heterosexual partners. Davis also studied his material with the electron microscope, and the findings prompted him to classify the causative organism as a *Corynebacterium*; its classification had been hitherto somewhat uncertain.

In a VD clinic in Pondicherry Lal and Nicholas (1970) collected 165 cases of granuloma inguinale between 1966–9, all proved by the finding of Donovan bodies. The age and sex pattern corresponded with that of the patients with other venereal diseases; moreover 92·7% of cases involved the genitalia, 9·7% the inguinal area and 7·3% the anal area; 45·5% had syphilis, 14·5% LGV and 1·2% gonorrhoea. Fifty sexual (presumably heterosexual) partners were studied; 26 had granuloma inguinale and 6 more may have had it. They conclude that all these findings support their theory of venereal transmission. It seems that the pattern of disease and perhaps its mode of transmission varies with the social and racial background.

Schistosomiasis

Schistosomiasis of the vulva in 3 African women is reported by Kingsley (1969), with histological demonstration of the parasites.

Amoebiasis

Gogoi (1969) reports 2 cases of amoebiasis, with trophozoite forms of *Entamoeba histolytica* found in the discharge; both patients had ulcers on the cervix, and one ulceration of the vulva also. Both had the parasite in the stools and responded to emetine. He speculates as to whether the apparent rarity of the condition in the genital area is due to failure to diagnose it or to high vaginal acidity.

Trichomonas and Candida

Although only *Candida* produces specific cutaneous lesions on the vulva as well as vaginitis, both may be considered, as non-specific vulvitis associated with trichomonas vaginalis (TV) is so common.

The details of culture need not much concern the dermatologist, but one may note that many papers have been written on the best methods to isolate both organisms. Thin *et al.* (1969) find wet smears as helpful as stained preparations for TV, whereas Hess (1969) prefers a wet preparation with or without culture rather than a stained preparation. Eddie (1968) however shows that, to find the maximum number of positives for *Candida*, the specimen must be cultured should the wet preparation be negative. Wasley and Rayner (1970) find that TV can be kept, if necessary, for months in liquid nitrogen in the presence of dimethyl sulphoxide. Oates *et al.* (1971) and Selwyn and Oates (1971) describe a new type of swab with a polyester sponge tip, which they think will provide excellent specimens with minimal discomfort, and enable large populations to be screened for the presence of gonorrhoea, TV or *Candida* as well as for cytology specimens. Presumably such swabs would also be of use in taking vulval specimens.

Many papers deal with the treatment of TV, with particular reference to whether or not resistance occurs to metronidazole and to alternative methods. McFadzean *et al.* (1969) found no difference between organisms from patients who seemed not to respond to the drug and from others who did, and Keighley (1971) found oral metronidazole highly effective when given and seen to be taken in a prison community. Benazet and Guillaume (1971) assert that although resistance can be induced in the laboratory, it is not a clinical problem. Evans and Catterall (1970, 1971) compare 2 new drugs with it and find metronidazole superior. They conclude that resistance does not occur but that factors militating against a cure include failure to take the drug, reinfection, inadequate absorption, and possibly inhibition of the drug by vaginal flora. Pimaricin is another drug which it is alleged has anti-TV and anti-*Candida* activity (Raab, 1969).

Cacciapuoti *et al.* (1969) claim that, with a new fermentation method, many different species of *Candida* can be rapidly distinguished, and confirm previous findings that, as well as *C. albicans*, species such as *C. guillermondi*, or *C. pseudotropicalis*, may be causal agents of vulvovaginitis.

Rohatiner (1966) has found that of 43 patients with clinical *Candida* infections in the genito-urinary tract, almost half had *Candida albicans* in the alimentary tract, compared with 27% of a control hospital population, and postulates that this is one important factor in causing relapse. He, too, stresses that *Candida albicans* is not the only pathogenic species. Oller (1969) gave amphotericin pessaries to check the proliferation of *Candida* which may follow treatment of TV with metronidazole; the *Candida* may not, however, cause symptoms.

The difficulty of distinguishing between carriage and active infection by *Candida* bedevils work on the subject. The criteria for distinction are not well defined, but the formation of pseudo-mycelium may be helpful (Rook *et al.*, 1968; Winner and Hurley, 1966), and the point is relevant to the much debated role of the contraceptive pill in *Candida* infection. The difficulty has been to study a control group, as well as all the variables in each group, such as the clinical significance of *Candida* (as opposed to its asymptomatic carriage), sexual activity, and so on. Davis (1969), with 1001 patients, found that infection with *Candida* occurred in 8·5% and was often symptomless. He did not find it was associated with the Pill. Diddle *et al.* (1969) compared 3179 patients on the Pill with 5240 who were not; they too concluded that the Pill had no aggravating effect. Rohatiner and Grimble (1970) came to the same conclusion. Catterall (1971), however, remains convinced that it is important. Spellacy *et al.* (1971) have made an extremely careful cross sectional study of women with an IUD and women on 2 types of contraceptive pill, and a prospective study of the same kind of group. The conclusion was that there was no increase of infection in those on the Pill. The authors point out that all workers have found more infection in those on the combined as opposed to the sequential type of pill. Kudelko (1971) suggests that allergy is a factor in *Candida* infections, as have other authors, e.g. Winner and Hurley (1966), and believes injection of *Candida* allergens may be helpful in vaginitis.

The occasional relation of *Candida* to more serious ills is stressed by Horowitz *et al.* (1969), who report *Candida albicans* septicaemia following rupture of tubo-ovarian abscesses; and by Ho and Aterman (1970), who describe microscopic evidence of *Candida* infection in a spontaneously aborted foetus. The last named authors believe that the infection was acquired *in utero*, and speculate that the abortion may have been caused by it, particularly in view of the fact that, in 6 of 9 cases of intra-uterine infection with *Candida*, labour was premature. Fox (1971) reports 5 fatal cases of superinfection with *Candida* (proved by post-mortem findings or blood cultures) in gynaecological surgery.

When nystatin and amphotericin and candidicin fail, dermatologists and gynaecologists may have recourse to gentian violet. Rosenkranz and Carr (1971), however, now warn that this is a potential carcinogen because it can interact with DNA in living cells; the work was carried out on strains of *Escherichia coli* which are deficient in an enzyme repairing damaged DNA.

Few, however, would agree with Beaton (1969), who believes that "an aggressive plan" in treating vulvo-vaginitis will succeed, and uses a pessary containing di-iodohydroxy quinone, sulphadiazoine and diethyl stilboestrol.

Pediculosis

Pediculosis pubis is reviewed by Fisher and Morton (1970) in England, and Altchek (1970) in the States. They agree on its increasing incidence, Fisher and Morton with evidence from records of 1954 and then of 1964–8, where the percentage incidence both relative and absolute rises steadily. They give a full account of the life history of the parasite, and treatment. They find its incidence lower in coloured immigrants than in the rest of the population.

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Society Proceedings

ROYAL SOCIETY OF MEDICINE

SECTION OF DERMATOLOGY

October 21st, 1971

Epidermolysis Bullosa Associated with Neoplasm of the Bronchus.—

Dr J. ALMEYDA.

A woman aged 60.

History.—She presented with mouth ulcers in February 1971. These failed to improve with many local applications including Oraldene, gentian violet and nystatin. Systemic therapy with ampicillin and oxytetracycline was also without effect. Blisters then developed on her lips, both mucosal and vermillion surfaces, buccal mucosa and also on the skin at sites of minor trauma.

On examination.—(April 1971). She had bullae on her hands and fingers, bridge of nose (site of glasses' pressure) and shins. Some were haemorrhagic. An intact bulla was present in the right buccal mucosa with ulceration on the gum margins. Many milia were present on sites of previous bullae. There was bilateral soft axillary lymphadenopathy and hepatomegaly to 5 cm below the costal margin. No abnormalities were detected on examination of her chest.

Investigations.—4.viii.71 (values on 24.viii.71 in brackets). Hb. 12·6 g/100 ml (11·4). E.S.R. 5 mm/hour (112). Electrophoretic strip normal (raised alpha 2 globulins). Serum proteins; 7·5 g/100 ml (8·1), albumin 4·5 g/100 ml (2·7), globulin 3·0 g/100 ml (5·4). Alkaline phosphatase 8·6 KA units (14·2 KA units). Antinuclear factor—diffuse positive 1 in 10. Porphyrins—stool, red cell and urine all normal. X-ray of chest normal on 4.viii.71 but showed an opacity in the apical segment of right lower lobe on 24.viii.71. Positive occult blood in the stools, but no abnormality was found in the gastrointestinal tract after proctoscopy, sigmoidoscopy, barium meal and follow through and barium enema, other than duodenal scarring of an old duodenal ulcer.

Histology.—Epidermis separated cleanly at basal line. No acantholysis. Fibrin and relatively few inflammatory cells seen in the space. No amyloid or PAS positive material seen.

Course.—Bronchoscopy was performed but a biopsy did not show any growth. Neoplastic cells were found in the sputum. On 1.x.71 a right lower lobectomy was performed and histology showed a pleomorphic squamous cell carcinoma. The resection was incomplete as growth involved the cut end of the resected bronchus and paratracheal glands. Palliative deep x-ray therapy was given, using Cobalt 60. Five treatments over a period of 6 days delivering a tumour dose of 2250 rads. The patient made an uneventful postoperative recovery although she still developed a few blisters. These were far less numerous than before the operation.

Comment.—Various bullous disorders have been recorded in association with neoplasia, namely erythema multiforme, dermatitis herpetiformis, bullous pemphigoid and epidermolysis bullosa acquisita. Of particular interest in this patient is the association with a rapidly growing carcinoma of the bronchus. This has not been recorded previously although lymphomata have been cited as a cause along with amyloidosis, myelomatosis, Crohn's disease and diabetes (Roenigk *et al.*, 1971).

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ROENIGK, H. H. JR., RYAN, J. G. & BERGFIELD, W. F. (1971) Epidermolysis Bullosa Acquisita. *Archs Derm.*, **103**, 1.

Addendum.— Auto-antibody Studies Using Immunofluorescent Techniques.—Sections of guinea-pig lip and carcinoma from patient showed no reaction with specimens of patient serum taken before and after operation for auto-antibodies to skin or tumour respectively in the indirect fluorescent antibody technique (Coon's method). Dilutions of patient serum used were 1 in 5, 1 in 10 and 1 in 20. " Wellcome " fluorescent anti-human immunoglobulin was used at a dilution of 1 in 15. (These tests were performed by Dr J. Perrin and Mr Bubel.)

Epithelioma Cuniculatum. — Dr Napier Thorne.

A man aged 51.

History.—First seen in September 1967 with 25-year history of wart beneath right sole. Over the years he had trimmed it himself and had chiropody. General health good.

On examination (1967).—Massive wart on right sole beneath metatarsal heads, 2·5 cm in diameter with hypertrophic collar and local tenderness.

Treatment and progress.—Curetted under local anaesthetic and healed in 6 weeks. January 1968 recurrence of wart at same site which was recuretted and healed slowly followed by relapse in October 1968 when recuretted and given radiotherapy 900 rad. January 1969; abcess appeared distal to treated area. Infection settled following oral tetracycline. March 1969; further curettage under general anaesthetic followed by formalin soaks proved unsuccessful in preventing immediate recurrence. September 1969; wide excision of affected area and skin graft. This had healed by January 1970. The following month further infection on border of scar. September 1970; pain in foot and wart recurred. October 1970; deep excision of area including plantar fascia with skin graft which healed slowly. June 1971; macerated skin on sole and discharge between 3rd and 4th toes. Onset of night cramps. Pain increased and became constant while infection was checked only by oral ampicillin.

On examination.—Walking with difficulty due to constant pain in foot. No inguinal lymphadenopathy. Tender indurated area on sole of right foot beneath metatarsal heads with small sinus between 3rd and 4th toes.

Histology.—March 1969. A curetted inflamed plantar wart covered with thick layer of partly parakeratotic, degenerate and macerated callus. October 1970. Low grade squamous cell epithelioma of the type referred to as carcinoma cuniculatum.

Comment.—In 1954 Aird *et al.* described 3 examples of a tumour of the forepart of the foot which gave rise to considerable difficulties in diagnosis. Clinically it was characterized by the presence of a soft bulky mass with extensive invasion of the soft tissues but little destruction of important structures and by the presence of numerous sinuses exuding foul-smelling greasy material. Pathologically the mass consisted of large and numerous anastomosing crypts lined by squamous epithelium and filled with breaking down keratin. The authors believed that the mass was a low grade squamous carcinoma, modified by extreme keratinization and the effect of pressure. One of their cases was a museum specimen. The other 2 required amputation because of extreme local invasion. No metastases were found. They coined the name epithelioma cuniculatum when the nature of the lesion was still uncertain, using the term " epithelioma " in its correct sense to imply a tumour of epithelium. Cuniculatum refers to the resemblance of the crypts to rabbit burrows. Thompson (1965) described 3 further cases in all of which the plantar lesions had first been diagnosed and treated as warts. All but one of the 5 clinical cases in these 2 papers were men. The youngest was aged 48 and the oldest 71. In several, initial biopsies had revealed nothing more sinister than wart tissue on microscopy. This was probably due to the biopsy having been insufficiently deep. Several hypotheses have been advanced as to the nature of these exceedingly uncommon tumours. In Aird's 2 cases no specific inclusion bodies were found which would be expected if they had originated as warts. Tumours of hair follicles and sebaceous glands can be ruled out as these structures do not occur on the soles. Neither do dermoid cysts appear on the feet. The final pathology is unmistakably that of a malignant neoplasm. Although plantar warts are usually considered to remain benign however long they have been present, the findings in my case and the small series of the other authors strongly favour an exception to this rule. Amputation would appear to be the treatment of choice if wide local excision fails. In the case of men, Symes amputation would appear to be indicated.

P.S.—Since the above meeting the patient has undergone a Symes amputation.

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THOMPSON, S. G. (1965) *Br. J. plast. Surg.*, **18**, 214.

The following cases will be published in *Proc. R. Soc. Med.*

Xeroderma Pigmentosum, Dr. A. KOBZA and Dr F. GIANNELLI (for Dr H. J. WALLACE).

Eruptive Basal Cell Papillomata with Underlying Mitotic Lesion, Dr J. A. WALTER (for Dr J. LEWIS and Dr T. W. E. ROBINSON).

Obituary

KENNETH MACGREGOR

The sudden death of Kenneth Penn Leslie MacGregor on January 15th, 1972, deprived the community of a dedicated doctor and humanist and the medical profession of a highly esteemed dermatological colleague.

Dr MacGregor was born in Hamilton, New Zealand, and was the third son of Mr and Mrs J. MacGregor, who was a descendant of the Penns of Pennsylvania. His father was killed in the first world war and after this the family moved to Hawke's Bay and Ken attended school at Waipawa and then Scot's College. He studied medicine at Otago University and graduated in 1938. He then became a member of the staff at Waikato Hospital in Hamilton and in 1940 married Muriel Chamberlain.

After serving as *locum tenens* for some general practitioners he went overseas in the Army Medical Corps and was for three years in Egypt. He returned to engage in general practice in Frankton, a suburb of Hamilton, New Zealand. After some years he left to study dermatology at St John's Hospital for Diseases of the Skin in London. Some eighteen months later he transferred his studies to the University of Pennsylvania following which he returned to set up as a consulting dermatologist in his home town. He was then appointed to the post of visiting dermatologist to the Waikato Hospital. Dr MacGregor was a foundation member of the New Zealand Dermatological Society and served a term as President. He was also elected to the membership of the Australasian College of Dermatologists.

His greatest interest outside his work and home was the breeding of horses. He loved and understood these animals and one of his stable ran in the Melbourne Cup last year.

His great outstanding characteristic was his unremitting devotion to the welfare of his fellow men. He was for a time president of " Heritage " and worked also for " Birthright ", two charitable organizations concerned with underprivileged children. He was also a member of the Cancer Society and for a long time of Rotary. Few associates knew of his generosity: that, for instance, he paid each year for Christmas dinner for a number of blind folk.

He was always a good companion at dermatological meetings and loved both the academic and social side of these. He was a good raconteur and would readily tell a joke against himself. It is a man of rare character who, although by no means a " Yes man ", had apparently no enemies; yet this was Kenneth MacGregor. We miss him greatly and offer our deepest sympathy to his wife Muriel.

M. J. PURDY

Br. J. Derm. (1972) **87**, 73

Correspondence

Psoriatic Alopecia

Sir,—A number of your readers have asked me to send you a summary of a paper which I read to the British Association of Dermatology in Edinburgh last June and which went by the title of "Psoriatic Alopecia". What I said was that contrary to the textbook view I have always found that hair-loss (hereinafter called alopecia) is commonly caused by psoriasis.

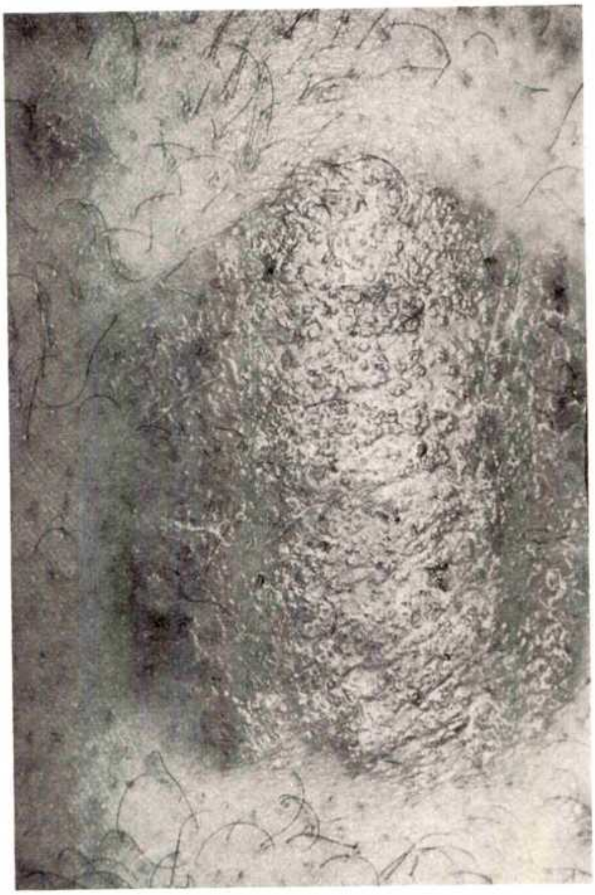

Fig. 1.—Alopecia in patch of psoriasis on leg.

My patients were male and female adults and children with various types of psoriasis, excluding the erythrodermic and pustular forms in which alopecia is well accepted. Whilst the main method used was that of clinical observation, in some instances hairs were counted in a unit surface area by marking a square of 0·5 or 1 cm sides on the skin surface and counting hairs directly in the lock issuing or cut from the area. Hair diameters were measured about 1 cm from the root end of hairs cut or plucked from both clinically normal and affected skin, using a graduated microscopic grid. Hairs were plucked in bunches of 50 or so and the roots examined microscopically. Skin biopsies

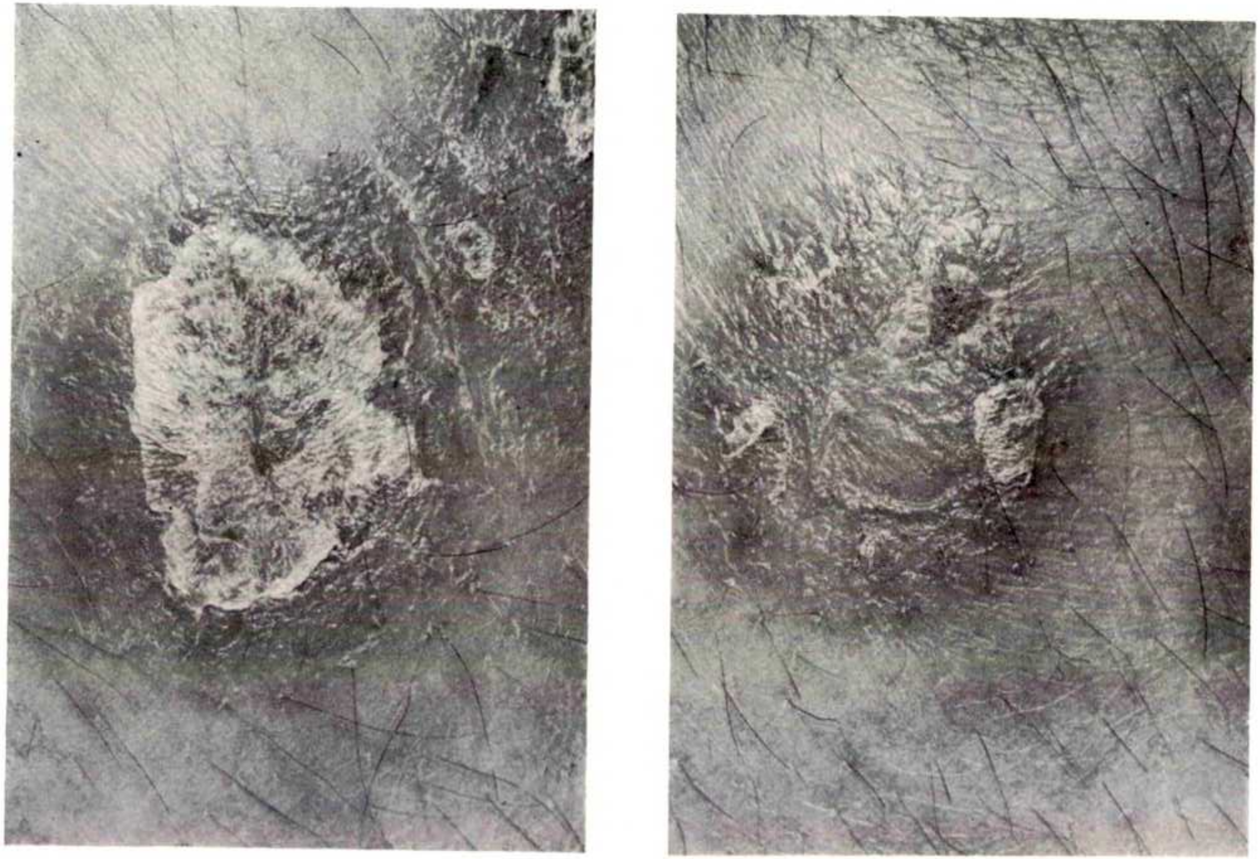

FIG. 2(a), (b).—Alopecia in fresh and untreated lesions of guttate psoriasis.

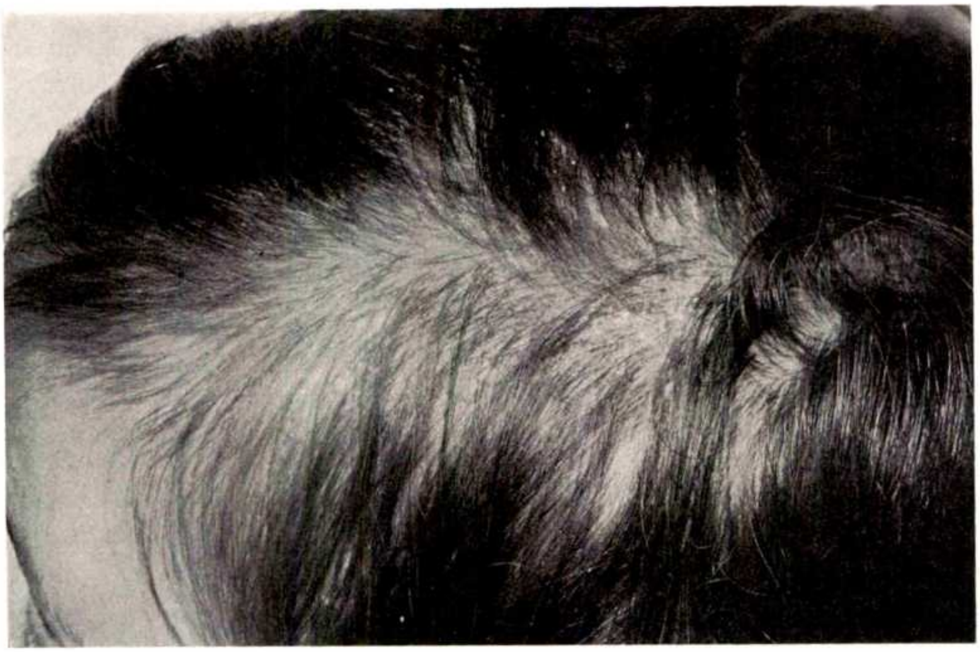

FIG. 3.—Alopecia of scalp in girl of 8 with recent onset of psoriasis. Hair loss is confined to the visible lesions.

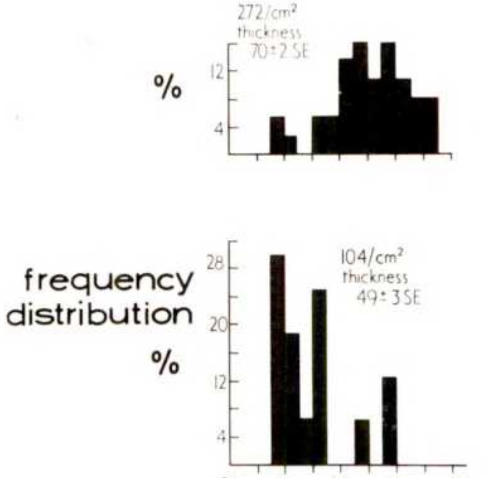

FIG. 4.—Hair density and histogram of hair thickness from clinically normal and clinically affected skin of patient shown in Fig. 3. The differences are easily seen in the actual lock of hair cut from the same surface area.

were prepared by depressingly conventional means except that some were cut parallel to the skin surface.

The findings were as follows:

(1) *Trunk and Limbs*

Most, but not all patches of psoriasis, on both trunk and limbs can be seen to have lost hair. This is shown in Fig. 1. This is not due to treatment because it occurs in untreated lesions as for example in a fresh crop of guttate psoriasis (Fig. 2a, b). The hair loss is evidently not due to scratching (though scratching speeds the process, and the stumps of broken-off hairs are often seen in the plaques on the limbs) (a) because of the too precise relationship of hair-loss to the lesions; (b) because hair-loss is also seen in lesions which have not been scratched or which are inaccessible to the desquamating digit. This alopecia is easily confirmed by counting.

(2) *Scalp*

Psoriasis also produces alopecia in the scalp and I have noted 3 types:

(i) Hair-loss confined to the lesions: this is the commonest type of psoriatic alopecia of the scalp. There is a very evident decrease in the density of hair in the plaques and the hair is often palpably and visibly finer (Fig. 3). This is easily confirmed by measurement (Fig. 4). There is too great a number of dystrophic bulbs in hairs plucked from the plaques (Fig. 5). When hairs are plucked to examine roots, the best yield is obtained when the hairs are pulled in the direction of the follicles. It was, alas, soon noticed that when hairs were pulled clumsily, that is to say against the direction of the follicles, they were easily snapped; moreover, the snapped hairs immediately curled-up, suggesting over-easy rupture of a poor shaft and internal realignment of its fibrous protein by stretch.

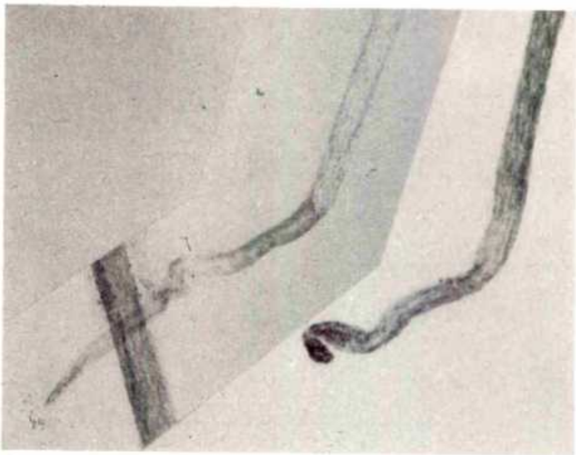

FIG. 5.—Two dystrophic bulbs from a patch of scalp psoriasis with alopecia. One shows early changes with twisting and thinning, the other shows advanced change and the bulb itself has become detached.

(ii) Acute hair fall: this has been seen in several patients and in 2 of these examined closely during the period of hair loss, many of the hair roots over the plaques were in telogen. Some of these patients also complained of a similar but less severe hair loss from the clinically unaffected parts of their scalp (Fig. 6).

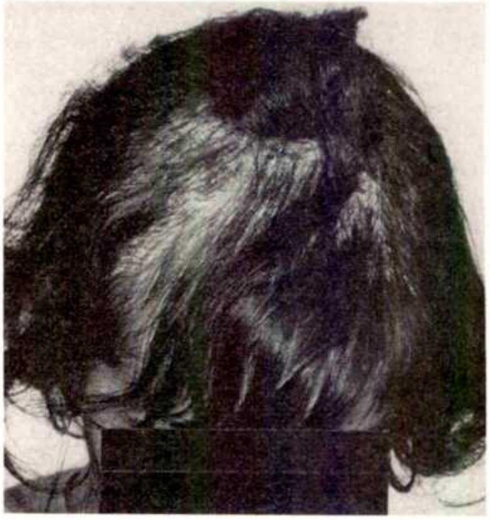
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FIG. 6.—This patient's alopecia was most marked in the patches but there was also some acute diffuse hair loss with many telogen hairs.

(iii) Destructive alopecia: alopecia with follicular destruction (alias "scarring alopecia") is the least common form of psoriatic alopecia. I have seen 6 patients with this condition in the last 2 years (Fig. 7 and 8). Histologically the scalp shows a paucity of follicles in some biopsies and perifollicular inflammation with destructional folliculitis and fibrous tissue "replacement" in others. The hair loss shows every appearance of permanency although neither the patients nor I are likely to live long enough to prove this.

There is little to discuss about these observations: hair-loss is a common feature of the psoriatic plaque: it is easily recognized and will easily be confirmed. Psoriasis affects the whole keratinizing epidermis and so it makes sense that it affects the nail (Dawber, 1971) and hair too (Wyatt *et al.*, 1971). As to mechanisms, the investigations I have done so far are as trivial as the observations, they suggest nevertheless that thinning of the hair associated with dystrophic bulbs is high on the list. I should also like to point out that the dystrophic bulbs of psoriatic alopecia do bear a resemblance to those seen in protein malnutrition (Bradfield and Bailey, 1969). Psoriasis also causes a true destructive alopecia (" scarring alopecia ") and although attempts will no doubt be made to rescue psoriasis from the black list of causes of destructive alopecia, time will show that it belongs there.

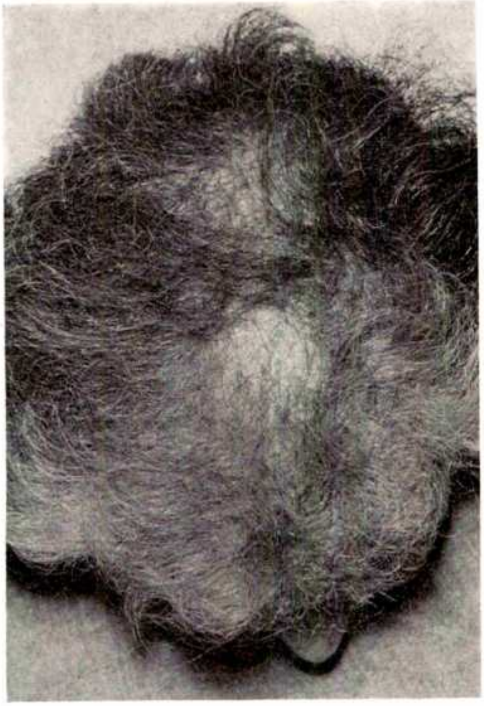

FIG. 7.—Chronic psoriasis with destructional alopecia. The scalp moved poorly over the skull and there was follicular destruction histologically.

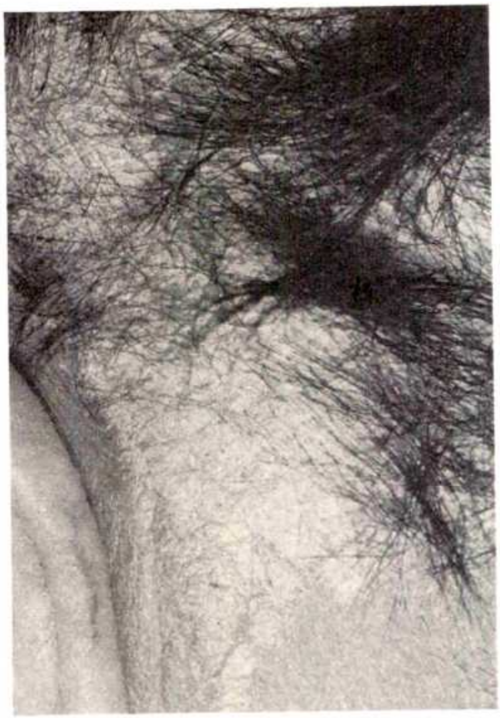

FIG. 8.—Chronic psoriasis with destructional alopecia shown histologically.

In summary, psoriatic alopecia is a commonplace and it only remains for it to be studied properly; indeed, the most interesting question about psoriatic alopecia is how we've managed to ignore it for so long.

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Pemphigus as an Autoimmune Disease

SIR,—We would like to comment on the paper " Is pemphigus an autoimmune disease " (Kreysel and Memmesheimer, 1971).

These authors, challenging the autoimmune nature of pemphigus, base their conclusions not on their own experiments but on data taken from the literature. We agree with the authors, though for different reasons, that the work of Grob and Inderbitzin (1967) cannot be taken as evidence of participation of pemphigus-type antibodies in the induction of acantholysis in rabbits. It should be mentioned, however, that Kreysel and Memmesheimer have erroneously quoted the course of the experiments as well as their interpretation, particularly when suggesting that pemphigus-type antibodies have been bound *in vivo* after the skin had been treated with sodium lauryl-sulphonate and dry ice.

In their paper Grob and Inderbitzin stated: " Even though the production of antiepithelial autoantibodies in rabbits has been possible and the autoantibodies were present in high titres in the serum, they were not present *in vivo* in the squamous epithelium containing the antigen ". In another paper (Inderbitzin and Grob, 1967) accompanied by photomicrographs showing marked acantholysis, no direct immunofluorescent staining was performed for the detection of the *in vivo* bound immunoglobulins at the same site. This would constitute a crucial argument for critical conclusions. The statement of Inderbitzin and Grob: " our experiments showed that acantholysis occurs in animals with high titres of antiepithelial autoantibodies provided that the barrier between epidermis and plasma is broken down, which otherwise prevent the antigen–antibody interaction and thus the occurrence of acantholysis " finds no confirmation in their experiments since they have not proven, or have not examined, the binding of these autoantibodies *in vivo*.

A comparison made by Kreysel and Memmesheimer between pemphigus antibodies and secondary autoimmune phenomena in heart and liver diseases or in skin burns is purely speculative. The skin burns mentioned by the authors, in which pemphigus-like autoantibodies can sometimes be transiently detected, may, in some cases, trigger off the development of true pemphigus (Chorzelski *et al.*, 1971). This may happen when the burned individual is particularly prone to autoimmune response. In these cases, in contrast to pemphigus-like antibodies, the antibodies are able to bind *in vivo*.

The cases were also cited in which pemphigus was precipitated by some drugs, in particular by butazolidine (Chorzelski *et al.*, 1966; Schuppli, 1967; Beutner *et al.*, 1970) and penicillamine D (Degos *et al.*, 1969; Hewitt *et al.*, 1971). In these instances, however, the relationship between skin lesions and the presence of antibodies provoked by a burn or long-term administration of some drugs cannot be proven unless the *in vivo* binding of these antibodies at the site of lesion is demonstrated by direct immunofluorescent methods.

Induction of acantholysis in experimental animals by passive transfer of pemphigus sera or isolated pemphigus antibodies is a direct proof of the pathogenic character of the antibodies (Chorzelski *et al.*, 1970; Wood *et al.*, 1972; Beutner *et al.*, 1972).

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Traction Alopecia due to " Hair Extension "

SIR,—Traction alopecia usually occurs in women, due to hairdressing styles or procedures which involve long periods of traction on the hair.

The complaint has been seen recently in a man, due to the process of hair linking known as hair extension. There are now centres where the procedure can be carried out in the major cities in this country, and the advertizing is directed at men with the implication that the man whose crown is well covered with hair is successful with women.

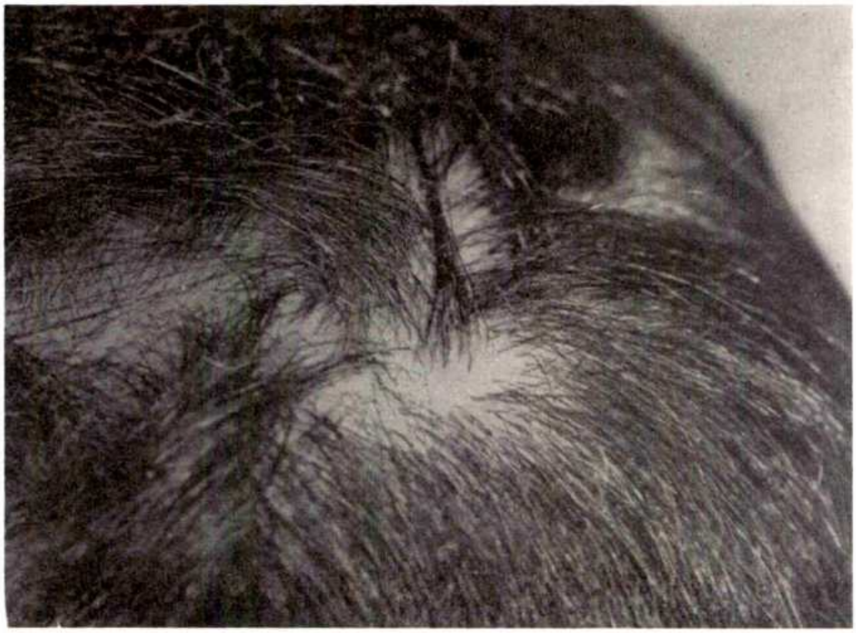

FIG. 1.—Traction alopecia at the base of a group of hairs used as a tight linkage to additional hair in the process of " hair extension ".

The method takes several hours and seems to be most suitable for those with moderately severe male type baldness. It is essential that the individual has some hair for additional wefts to be linked to. The wefts are made of rows of matched hairs tied to three strands of brown cotton. In addition to the wefts, a polyethylene mesh may be used to cover larger areas, with extra hair tied to the mesh. If it is cosmetically necessary to keep the wefts and mesh very closely adherent to the scalp as in the frontal region, an adhesive is used as well.

The wefts are joined to existing hair by grouping a number of hairs into a workable thread, so that these can be tied to the wefts (Fig. 1). Many such groups of hairs may be gathered to tether the wefts, and when the replacement of hair is complete there is uncomfortable pulling on these groups of hairs. They stay tight for several days, and it is this traction that has been seen to cause small areas of alopecia.

The cosmetic effect of the method is excellent. The linked hair can be shampooed, and lightly combed or brushed, and is certainly almost undetectable. But because of natural hair growth adjustments have to be made to correct loosening about 5 times a year.

The initial outlay for an individual is about £200, and for each adjustment he may expect to pay about £7.

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R. R. M. Harman

Innervation of Sweat Glands in Horses and Dogs

Sir,—We were most interested to read the report by Bell and Montagna (1972) in *Br. J. Derm.*, **86**, 160 on the " Innervation of sweat glands in horses and dogs " since we have been working on similar problems in the beagle. Our approach, however, has been physiological and pharmacological rather than anatomical and histochemical. Using an electrolytic water analyser system (Meeco) for the continuous recording of sweating we have studied the responses elicited on the hairy surface of the dog by local pharmacological stimulation, electrical stimulation of sympathetic nerves and electrical stimulation in the hypothalamus.

We find that the sweat glands respond to a wide range of compounds including acetylcholine and α and β adrenergic drugs, and that they can also respond to preganglionic, postganglionic and direct ganglionic electrical stimulation. This work is currently in press for the *Journal of Investigative Dermatology*. Preliminary results also indicate that electrical stimulation of the hypothalamus in beagles will also produce sweating on the hairy skin of these animals. As yet we have not mapped the hypothalamic regions but work is in progress towards this end.

Bell and Montagna report that the sweat glands in the horse showed cholinesterase activity, although it is reported that these glands react to circulating adrenaline. This is similar to the situation in the human where eccrine sweat glands appear to be cholinergically innervated although they will respond to adrenaline and isoprenaline (Warndorff, J. A. personal communication) as well as to acetylcholine. The extreme situation seems to be found in the hairy surface of the dog where Bell and Montagna could not demonstrate any innervation but where we have seen clear responses to peripheral and central nervous stimulation.

Certainly the sweat glands of the dog, the horse and man show more functional similarities than was previously thought, if only in terms of their complexity of activation.

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The Pathogenesis of Lichen Planus

SIR,—In the review article on this subject, Dr Black (1972), mentions that little supporting evidence exists for the hypothesis that lichen planus is caused by an auto-immune reaction. We can offer some preliminary results in support of this mechanism.

Peripheral blood lymphocytes from 6 patients with the diagnosis of lichen planus were cultured in the presence of a homogenate made from a lesion taken from the patient's own skin. Lymphocyte transformation in response to the skin homogenate was obtained in 2 of the 6 cases and this was confirmed by an increased uptake of ^{3}H thymidine. Some spontaneous transformation was noted in control cultures in 2 cases, but this was not accompanied by increased transformation in response to the addition of skin homogenate. These cultures also showed an abnormally high uptake of ^{3}H thymidine. In the remaining 2 cultures, no transformation was noted. The response of 3 normal subjects' lymphocytes to their own skin *in vitro* was negative.

As these results show no clear-cut pattern of response, a larger series will be required and in the meantime no statistically reliable conclusion can be drawn. However, the available evidence is compatible with the idea that lichen planus is an auto-immune disease. The nature and origin of the antigenicity of the skin is obscure. It could originate in the skin itself or by the adsorption of low molecular weight compounds, such as drugs, to the skin. Alternatively, it could result from adsorption of bacterial or viral products from infection anywhere in the body or as a result of the presence of antigenic determinants produced by the epidermal cells in response to a cryptic virus infection.

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Use of CIBA 1906 (Thiambutosine BP) in Vitiligo

SIR,—It is agreed that a safe, effective and easily obtainable local or systemic preparation is required for the treatment of vitiligo.

CIBA 1906 is used in the treatment of leprosy and is presented as 0·5 g tablets. In 1970, following earlier reports from Turkey and Mexico of CIBA 1906, of its usefulness in vitiligo, particularly if used in combination with u.v. light or sunlight, I obtained supplies of the drug for a pilot study in this condition. Dr Stanley Browne, Director of the Leprosy Study Centre, confirmed its safety and had noted that a small percentage of patients with leprosy treated with it did show either increased pigmentation of hypopigmented areas or increased pigmentation generally. However, he did not anticipate a beneficial response in vitiligo.

Ellard (1961) showed that, in man, only about 10% of oral doses of thiambutosine of up to 1·5 g are absorbed and that larger doses do not result in increased absorption. Maximum absorption can be achieved by giving 1·5 g three times daily. The drug is rapidly and completely metabolized after absorption.

I have been using CIBA 1906 since late October 1970 and have treated 5 white patients with vitiligo of from 14 months to 4 years duration. The age range of the patients (3M 2F) was 22–49 years and the distribution of lesions was widespread in one patient, upper and lower limbs in another, face and other areas in the remainder. Patients have received the drug for periods varying from 5–12 months. The usual dosage regime has been a single dose of 2 g followed within 30 min by exposure to E_1 doses of u.v. light, the latter being given 2–3 times weekly. The patient with widespread vitiligo took 3 g prior to u.v. light and some patients took 0·5 g four times daily in the summer relying on natural sunlight.

Results have been disappointing in that none of the patients has shown any clinical improvement. No side-effects have been experienced. I do not think that further evaluation of this drug in vitiligo is warranted.

Department of Dermatology
St Bartholomew's Hospital
London E.C.1

JULIAN VERBOV

REFERENCE

ELLARD, G. A. (1961) The Absorption, Metabolism and Excretion of 1-(p-dimethylamino phenyl)-3-(p-butoxyphenyl) -2-thiourea in Man. Part 1. A Study Using Colorimetric Methods. *Lepr. Rev.*, **32**, 233.

Br. J. Derm. (1972) **87**, 83

Books

Current Problems in Dermatology Vol. 4. Edit. J. W. H. MALI (1972). Basel: Karger. Pp. 230, 62 illus. Price £10.60.

This book contains a series of articles on almost totally unrelated topics and this in itself is a weakness. The first subject, " Exfoliative Dermatitis (Erythroderma)" by J. E. Adam, Ottawa (18 pp.), is a workmanlike review of all aspects—clinical, pathological and therapeutic, of this difficult clinical state. Considerable emphasis is laid on the numerous known precipitating factors and the recent literature on the associated general abnormalities of homeostasis is adequately discussed.

This is followed by " Cutaneous Manifestations of Toxoplasmosis ", J. Justus, Dresden, which occupies 19 pages. It is a good review of the condition and the literature. A figure of 50% of latent infections in the total population is quoted of which one per thousand becomes manifestly sick. It seems doubtful if this is applicable in this country at least. Since " the occurrence of skin lesions is rather rare " it hardly seems justifiable to include the topic as a current problem in dermatology.

The third contribution " The Acid Mucopolysaccharides of Skin " (P. D. Mier, Nijmegen) 25 pp. is a down to earth account of the chemistry, biology and clinical significance of these very important substances. A table listing current nomenclature with synonyms will be helpful to many. The account is written clearly, making the subject apparently simple. There is an excellent critique of methods of assaying the AMPS which points out pitfalls into which many have fallen and are likely to continue doing. This section could be extremely useful to the investigative dermatologist and to the clinician who wishes to gain some background information on mucopolysaccharides in skin.

Klaus Wolff, Vienna, contributes a masterly review of the " Langerhans Cell ", 57 pp. This covers every aspect of this enigmatic cell and is certainly the best account of this topic in the literature at the moment. If the book were slightly cheaper it would almost be worthwhile buying it for the sake of this review. The account is strictly limited to factual observation and is beautifully illustrated by his own electronmicrographs which are very well reproduced. Only after timely cautioning in relation to the interpretation of the ATP-ase reactions in human and guinea-pig cells and of the significance of the Langerhans granule does the author permit himself a few cautious comments as to their possible roles.

" Tolerance and Desensitization in Experimental Eczema " is discussed by L. Polak and J. R. Frey, Basel, 26 pp. This is an interesting section concerning a topic which has not received the attention which it deserves. Methods of inducing tolerance in guinea-pigs are well documented and the theoretical implications clearly discussed. The main emphasis is upon hypersensitivity to chemical haptens (Type IV reactions). The induction of tolerance is rightly distinguished from desensitization. This section fulfills a useful purpose in bringing together the scattered literature on this subject.

" The Role of the Basophil Leucocyte in Hypersensitivity ", by P. Wolf-Furgensen, Copenhagen, is disappointing (17 pp.). In several instances the author reveals his ignorance of modern concepts of hypersensitivity responses. There is considerable emphasis upon the older literature and a neglect of several recent studies in the English language. The English is quaint and at least one reference in the text " Kirshbaum *et. al.* 1967 p. 190 " does not appear in the list. A redeeming feature is the fact that the occurrence of early local basophilia in delayed hypersensitivity—an observation made by the author and since confirmed—is well illustrated and discussed. The phenomenon has received insufficient attention.

The final section on " Dermabrasion " 25 pp. by E. Epstein, Oakland, is well worth reading. The dry humour which pervades the pages suggests that the author should perhaps try writing for a larger public. In fact it gives a very balanced and sane account of methods, virtues and limitations of the technique which can be summarized as " It has some applicability in properly selected individuals treated by experienced facile operators ".

In general the inherent defect of this publication is that it contains too wide a variety of topics to appeal to any one individual. Spelling errors by English or American standards are common and occasionally amusing. Larger institutes could consider acquiring it as some of the contributions are undoubtedly of interest to specialists in those particular fields.

C. N. D. CRUICKSHANK

Mémento Immunologique de la Sarcoidose. J. CHRÉTIEN and J. C. SALTIEL (1971). Paris: Masson. Pp. 111. Price £3.10.

The solution to the problem of sarcoidosis and the sarcoid reaction is likely to lie in the field of immunology. At times it seems very close; but immunological will-o-the-wisps are a common fact of life. The work of Jones Williams in this country and, paradoxically, the current Kveim controversy, have provided a renewed stimulant in a direction that should certainly be rewarding.

Immunological studies have traditionally shown abnormalities in four respects but in only two of these—suppression of delayed-type sensitivity and the Kveim reaction—have they been at all consistent. The extent and significance of changes in humoral antibodies and plasma proteins have been less well-established.

It is particularly appropriate at this juncture, therefore, that the very considerable literature on the immunology of sarcoidosis should have been collected together in this concise and well-presented monograph. The 404 quoted references brings us up to the paper of Israel and Goldstein in 1971. Though any précis of immunology is virtually out-of-date as soon as it is written, this little book will remain for some years a most useful reference to the accumulated work in this field. It is warmly recommended to all dermatologists interested in this fascinating disease.

D. S. WILKINSON

The Pathologic Anatomy of Mycoses. Edit. R. D. BAKER (1971). Berlin: Springer. Pp. xiv + 1191, 796 figures, two in colour. Price £61.20.

This book, published in English, is the third volume of " Handbuch der speziell en pathologischen Anatomie und Histologie ". Not withstanding its title it is intended by the editor of the series to be a " complete hand-book on human infections with fungi, actinomycetes and algae including fundamentals of human mycology, taxonomy, clinical aspects, epidemiology and pathological anatomy ". It is sad to say that this high aspiration has failed; it is an extraordinarily disappointing book.

The first and most serious criticism is the almost complete lack of editing which has resulted in a rambling, repetitious, ill-organized text. For example the mycology of the fungi causing some of the deep mycoses is covered in Chapter 2 by Jan Schwartz. Most of this chapter is repeated at length, but with some minor variations, in the subsequent chapters on the different deep mycoses. Even Dr Schwartz himself when dealing with histoplasmosis has felt obliged to repeat the mycology and in this case to use at least one illustration which he has previously included (figure 9 on page 21, figure 2 on page 70). However, the mycology of the dermatophytes is not considered anywhere in the text.

The section of African histoplasmosis is divided into two parts but the two authors who cover this disease deal with essentially the same material and each section includes a full account of the mycology of *Histoplasma duboisii*; this has of course already been included in chapter 2! Some contributors include notes on treatment, others do not.

The general lay-out of the chapters varies considerably, the order of topics covered being different in different sections; sometimes, for example, epidemiology precedes mycology, sometimes it follows it. There are also considerable variations in the type used for the sub-headings of the different sections. Some of these are indicated by numbers, some by capital letters. The level of referencing varies widely between the different contributors. All references include titles, but as they are set out as continuous type, not in separate columns they are difficult to read. The index of authors seems accurate and therefore valuable, but the subject index is far too brief. Of the first ten subjects I tried to find, five were missing.

For those dermatologists whose main concern is the superficial mycoses, there is a lengthy section (170 pages) by J. H. Graham and C. Barroso-Tobila. This consists mainly of their considerable personal experience of the histopathology of ringworm infections. It is beautifully illustrated. Anyone making a special study of this subject will no doubt be prepared to peruse this section carefully, but for the more general reader there is very little of value in it, and someone new to the subject will find this a very confusing account. Curiously the authors adopt the taxonomic nomenclature of F. Blank as set out in a paper of 1959. They seem totally unaware that perfect states of many dermatophytes have been discovered and their nomenclature of the species is, therefore, not surprizingly full of anachronisms. This is high-lighted by a small section, by other authors, later on in the book entitled " Arthrodermosis " and entirely devoted to infections with *Arthroderma simii*! This reviewer found the use of abbreviations such as " S.C. " and " S.G. ", introduced without explanation an irritating feature. They turned out to be " stratum corneum " and " sebaceous gland "; he is still puzzling over some others.

In the section on pityrosporum species, pityrosporum folliculitis is introduced as a new entity; a personal contribution from the authors based on rather scanty evidence. Seven pages are devoted to erythrasma, surely now well enough established as a bacterial infection to be excluded from this volume. The references in this chapter are rather fewer than average for the book, and many important papers are conspicuous by their absence.

Superficial candiasis is mentioned only briefly by these authors. They refer the reader to Professor Winner's chapter on candidiasis in which superficial and deep infections are covered, with the emphasis on the latter. Once again there are beautiful illustrations but only 12 full pages of text. In a book running to nearly 1200 pages this would seem to be rather poor rations for such an important subject. Extraordinarily there is no account of candida as a commensal organism and the fundamental concept of the shift from commensal to parasite is largely ignored. Statements such as, " superficial (candida) infection is contracted as a result of direct contact with infected persons " and " infection of the gut frequently follows ingestion of infected saliva ", are misleading to say the least. The discoveries of defects in delayed type of hypersensitivity in chronic mucocutaneous candiasis are not discussed, though this is perhaps more understandable as the work is fairly recent.

There are of course valuable sections in this volume especially Professor Baker's own Mucormyocosis, Chick's chapter on North American Blastomycosis and Huntingdon's Coccidoidomycosis. The last in spite of its almost anecdotal style is well worthy of attention.

Unfortunately owing to its bad organization this book is very difficult to use as a work of reference and in some sections is surprisingly incomplete compared with its smaller much less expensive competitors. For those unfamiliar with the general principles of this subject especially for dermatologists in training it must be said that the section on the superficial mycoses is confusing, out of date and often inaccurate. It cannot be recommended. At the extraordinary price of £61.20 this is not a book for the personal bookshelf, nor for the small departmental library. Even those in charge of larger hospital libraries should be reminded that for less than half this sum one can purchase a handful of reliable standard texts on medical mycology all in recent editions.

S. O. B. Roberts

Current Concepts of the Histology of Oral Mucosa. (Proceedings of a Symposium held at the University of Illinois College of Dentistry.) Edit. C. A. Squier and J. Meyer (1971). Springfield: Thomas. Pp. 319, illus. Price £8.00.

The limitations implied by the title of this book should not be allowed to discourage the prospective reader. He should also, incidentally, not be misled by the title on the spine of the dust jacket (" Current Concepts of the History of Oral Mucosa ") which is singularly unhelpful.

The book is the result of a Symposium by 26 contributors held at the University of Illinois and is divided into 3 sections i.e. epithelium, connective tissue and physiological aspects. There is virtually nothing of histology in the usually accepted sense of the word. The emphasis is upon ultrastructural anatomy and covers the normal features, keratinization, tonofibrils and kerato-hyaline granules, dendritic cells, the basal lamina and cells of the lamina propria including inflammatory cells. These electron micrographs are of a high standard and well reproduced.

Among other features of epithelia that are discussed are the regional variations, biochemistry and cell renewal. With respect to connective tissue, the relationships between the structure and function of the masticatory and lining mucosae are compared. There is also a brief chapter on connective tissue ground substance. Physiological aspects of the oral mucosa embrace studies of the circulation, including electron microscopy, innervation, and some investigations of the permeability of this tissue.

The structure and function of the oral mucosa as a whole are therefore fully examined and though there is inevitably some variation in the quality in the chapters by different authors the overall level is very high. The references too are numerous and helpful. The book as a whole is most informative, interesting and a pleasure to read.

Regrettably knowledge of the biology of the oral mucosa is a long way behind comparable knowledge about the skin. In mitigation it must be said that it is probably a more difficult area to investigate. This book, however, goes a long way to filling a much needed gap and the editors' aims as stated in the preface i.e. "... to provide teachers and research workers in oral histology and the clinical fields in which it is applied, with a coherent account of current knowledge of oral mucosa", are amply achieved.

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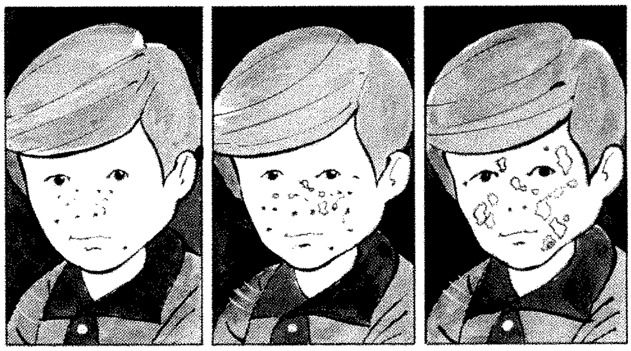

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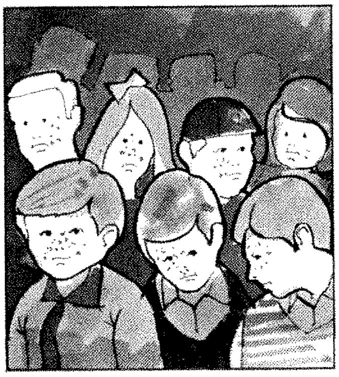

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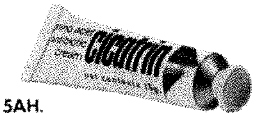

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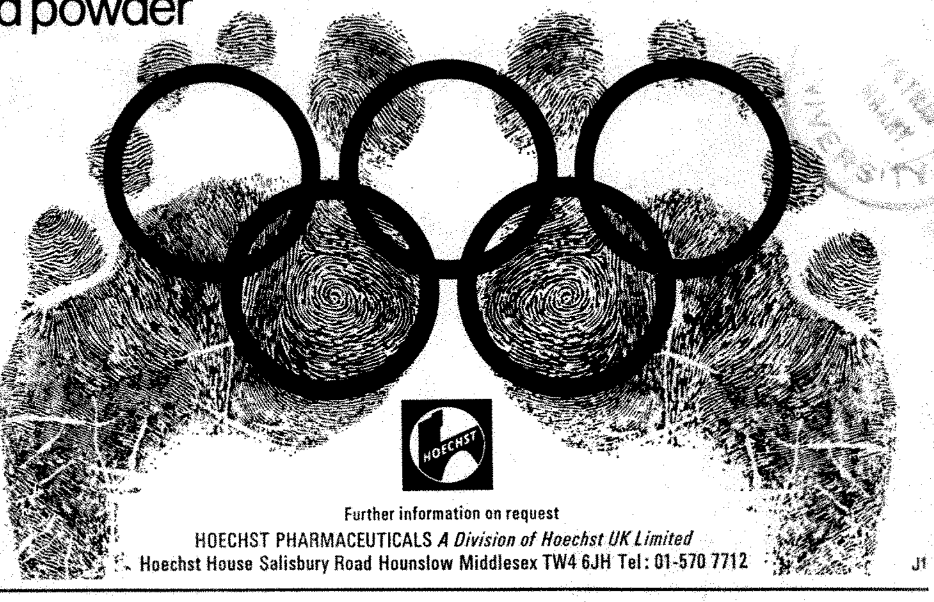

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CONTENTS (*Continued*)

Society Proceedings

Royal Society of Medicine, October 21st 1971 70

Obituary

Kenneth MacGregor 72

Correspondence

Psoriatic Alopecia
Sam Shuster 73
Pemphigus as an Autoimmune Disease
T. Chorzelski and S. Jabłońska 78
Traction Alopecia due to " Hair Extension "
R. R. M. Harman 79
Innervation of Sweat Glands in Horses and Dogs
D. W. K. Cotton and P. van Hasselt 80
The Pathogenesis of Lichen Planus
I. Sarkany and P. M. Gaylarde 81
Use of CIBA 1906 (Thiambutosine BP) in Vitiligo
Julian Verbov 81

Books

Current Problems in Dermatology Vol. 4. (Mali (Edit.)); Mémento Immunologique de la Sarcoidose (Chrétien and Saltiel); The Pathologic Anatomy of Mycoses (Baker (Edit.)); Current Concepts of the Histology of Oral Mucosa (Proceedings of Symposium at Illinois) . 83

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

Senear-Usher Syndrome and Internal Malignancy
N. K. Saikia and L. E. S. MacConnell 1
Allergic Contact Dermatitis to Garlic
E. Bleumink, H. M. G. Doeglas, A. H. Klokke and J. P. Nater . 6
The Effect of Enzyme Detergents on Transepidermal Water Loss from Normal Skin
Rowan Adams, C. L. Hales and E. J. Raffle 10
Human Epidermal Cell Cycle *in Vitro*
D. P. Chopra and B. A. Flaxman 13
Combined Surgical and Chemotherapeutic Treatment of Fortner's Amelanotic Hamster Melanoma AMel 3
E. Haneke, W. Wohlrab and J. Peker 18
The Adenosine Triphosphate Content of Guinea-pig Skin Stored at —196°C
S. A. Carney, M. Hall and C. R. Ricketts 23

Pharmacology and Treatment

The Effect of Glycerol on the Percutaneous Absorption of Methyl Nicotinate
Jonathan Hadgraft, J. W. Hadgraft and I. Sarkany . . . 30
The Effect of Clindamycin in Acne—a Clinical and Laboratory Investigation
W. J. Cunliffe, J. A. Cotterill and B. Williamson 37
Treatment of Pityriasis Rubra Pilaris with Azathioprine
G. A. Hunter and I. J. Forbes 42

Case Reports

A Benign " Malignant " Ulcer
P. C. H. Newbold and R. B. Stoughton 46
Unilateral Spider Naevi
W. J. Cunliffe, B. Dodman and M. J. Butterworth 51

Comment

Electron Microscopy: a Functional Approach
J. A. A. Hunter 53

Review

A Review of the Recent Literature on Diseases of the Vulva—Part II. Vulvitis: Infections
C. M. Ridley 58

(*Continued on* p. iii *of cover*)

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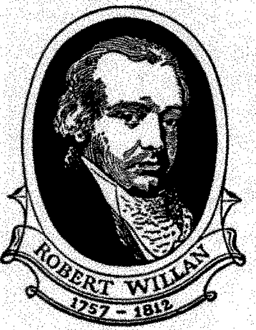

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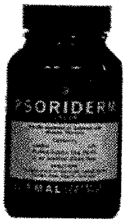

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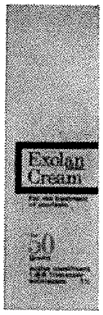

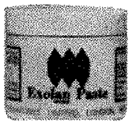

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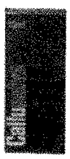

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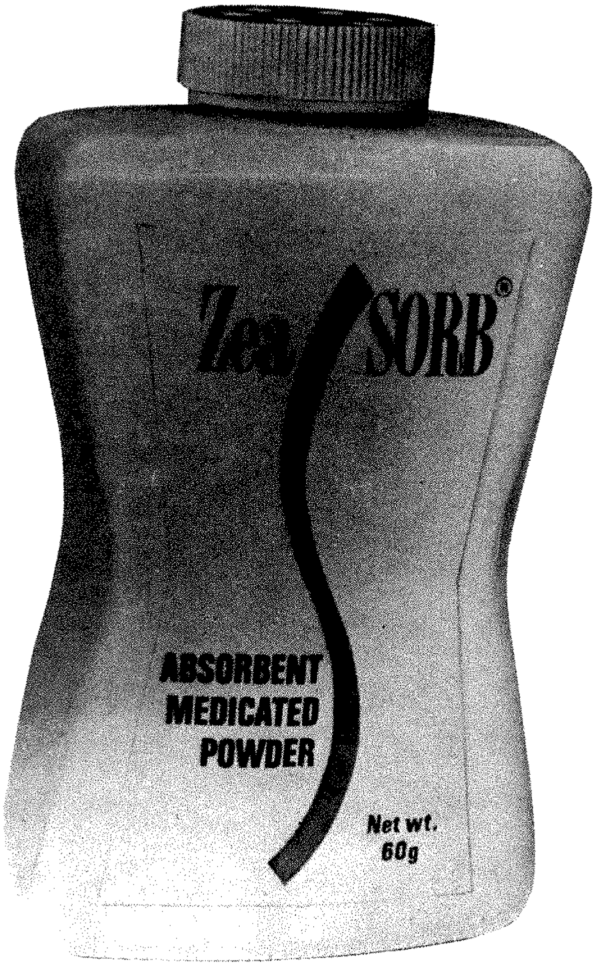

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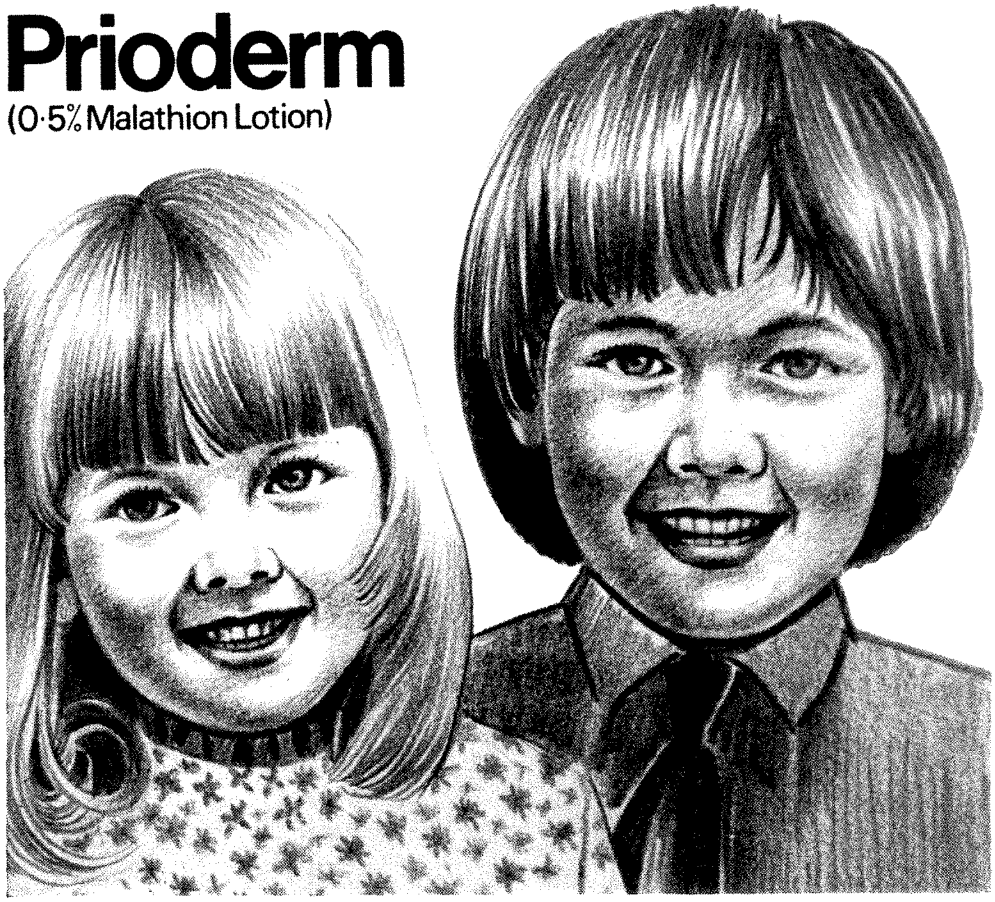

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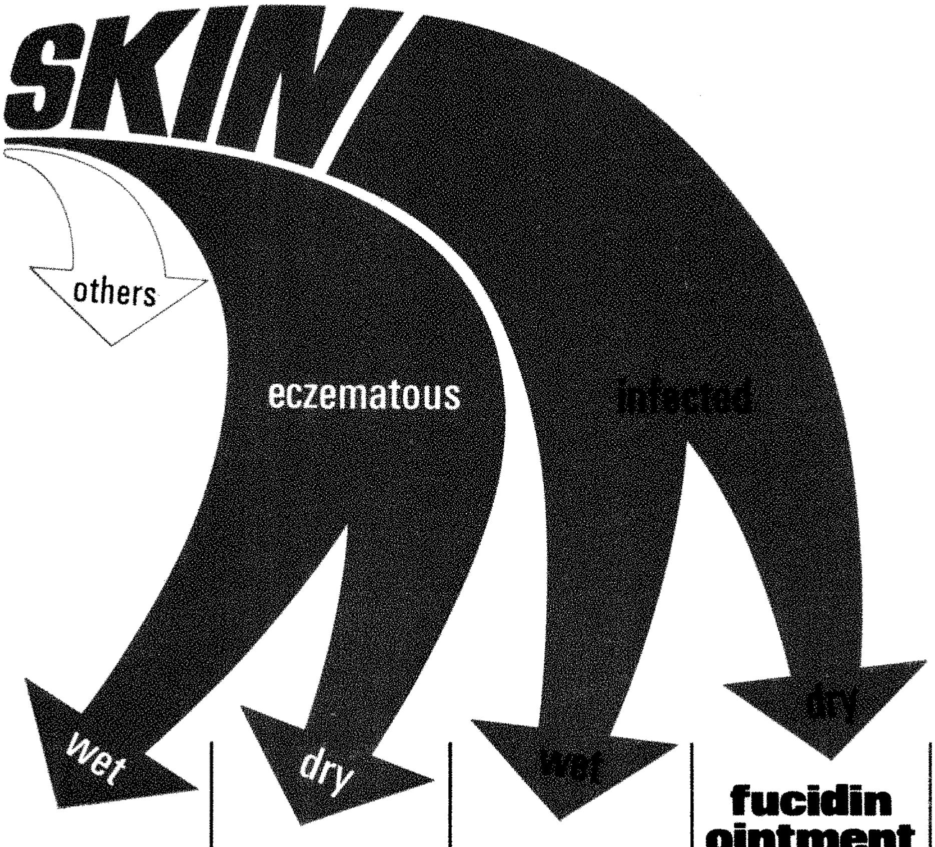

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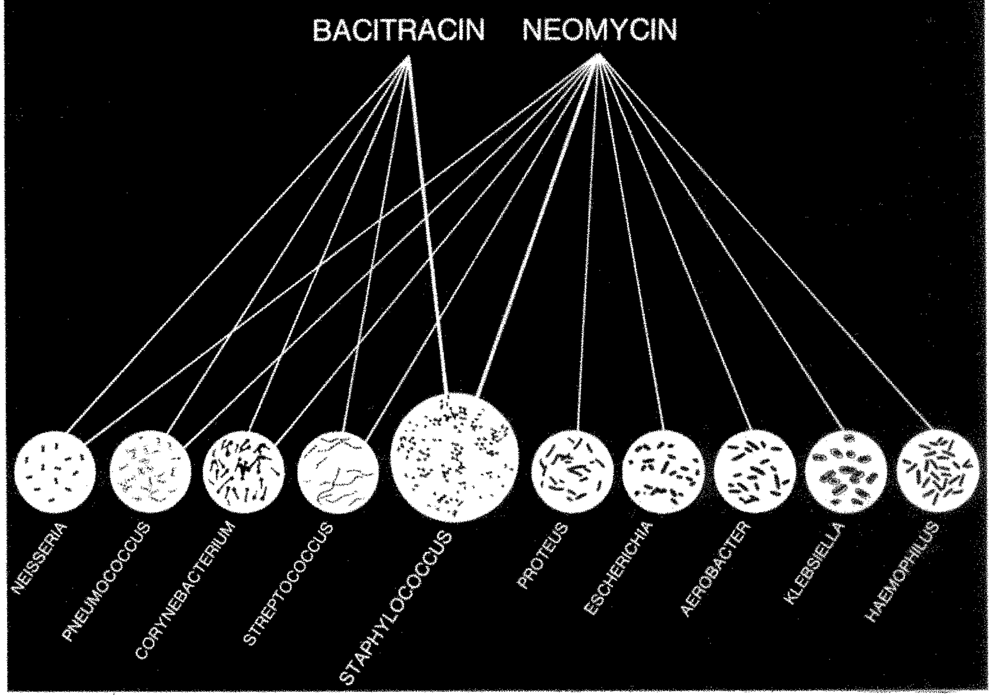

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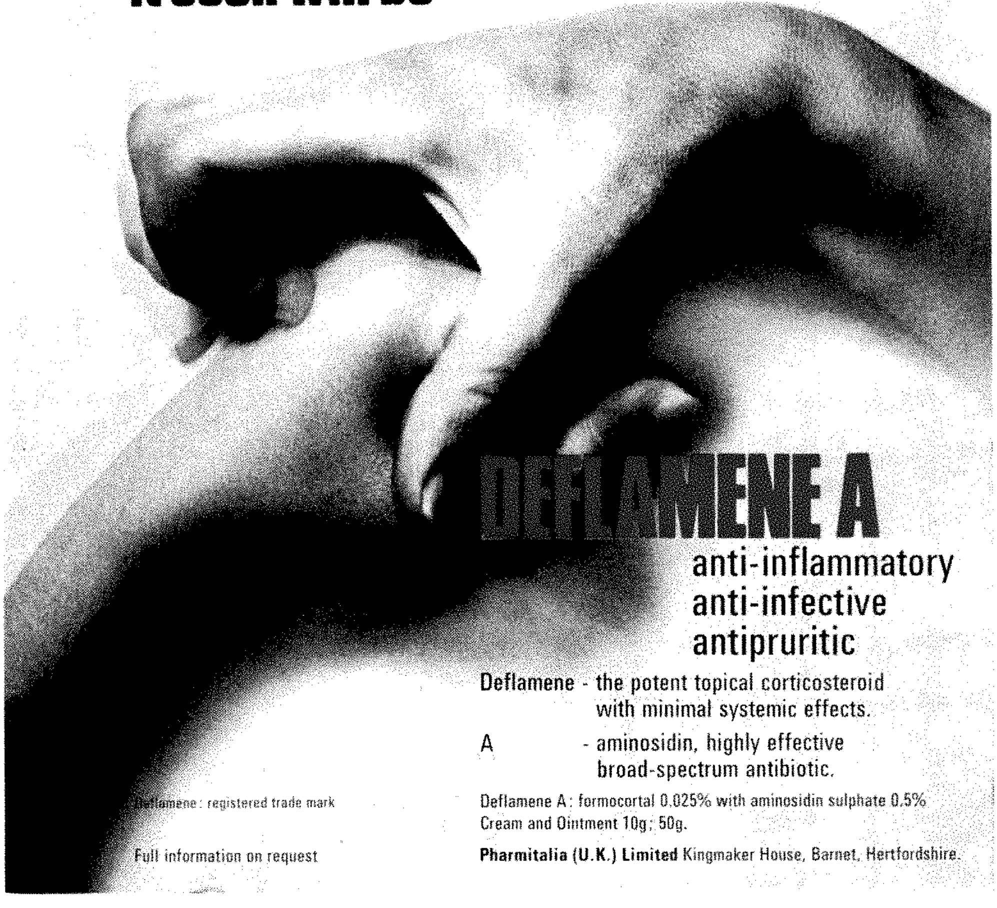

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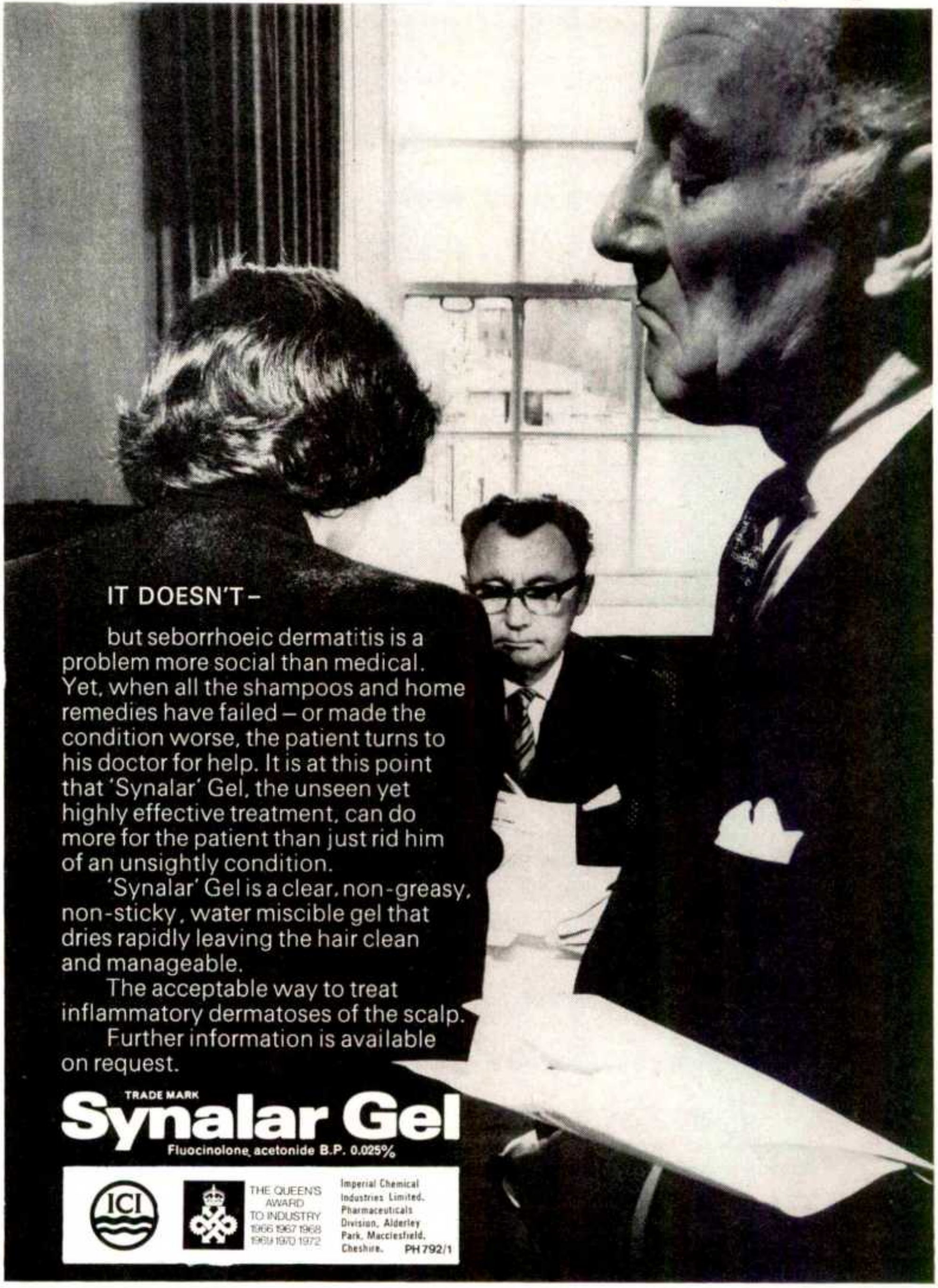

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CUTANEOUS POLYARTERITIS NODOSA

PETER BORRIE

Summary.—The case histories of 102 consecutive patients suffering from polyarteritis nodosa were reviewed. The 17 patients with skin lesions were personally examined and investigated. Thirteen were found to have polyarteritis affecting the skin. In one of these cutaneous involvement occurred only 4 days before death and 5 weeks after the onset of the illness. The other 12 showed remarkably similar clinical and pathological features and, together with 3 cases from another hospital, are described in detail.

Only the skin, skeletal muscles and peripheral nerves were involved. No deaths have occurred to date (longest follow-up period 23 years), and the disease is now either entirely quiescent or represented only by occasional cutaneous lesions.

It is concluded that about 10% of all cases of polyarteritis nodosa, and 30% of those proven histologically, are of this benign type and that this variety can be easily recognized clinically.

THE question as to whether there is such a condition as cutaneous polyarteritis nodosa has been debated at least since 1931 (Lindberg). There appear to be 4 main reasons why a definite conclusion has never been reached.

First, some authors (e.g. Melcer and Venkei, 1947) accepted as cutaneous polyarteritis nodosa any case in which the skin was involved, irrespective of the type of lesion. Since these cases covered virtually the whole spectrum of the malady, this approach to the problem led to the conclusion that cutaneous polyarteritis nodosa differed in no way from the non-cutaneous form.

Secondly, some reports failed to lay down rigid histological criteria on which the diagnosis of polyarteritis could be based. Thus any cutaneous lesion showing the microscopic changes of a vasculitis was accepted as an example of polyarteritis nodosa (e.g. Alkiewiez, 1933; Miescher, 1946). Many of the cases described in these papers can now be easily recognized as examples of other types of vasculitis, the commonest being allergic vasculitis (leucocytoclastic angiitis).

Thirdly, there were those papers which described both the clinical and histological appearances of the skin lesions in great detail, but failed to mention either the presence or absence of systemic involvement, or the course of the disease (e.g. Ruiter, 1958). Thus, it is not possible to decide whether such cases differed materially from polyarteritis nodosa without cutaneous lesions.

Finally, cases have been described over the years with the classical histological picture of cutaneous polyarteritis nodosa, in which the skin was predominantly involved and in which the disease was chronic and the prognosis relatively good (Lyell and Church, 1954; Fischer and Orkin, 1964). The inferences drawn from

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such cases, however, varied. Lyell and Church, for instance, as a result of a study of 3 cases, 2 cutaneous and one systemic, suggested that there were 2 forms of polyarteritis nodosa, cutaneous and visceral. On the other hand, Fischer and Orkin, also after studying 3 cases, came to the conclusion that there are not 2 separate entities and that cutaneous involvement merely varies inversely with the severity of the visceral lesions. More recently, Golding (1970) described 3 cases of polyarteritis nodosa confined to the skin or leg muscles and pointed out that, in these circumstances, the disease often follows a relatively benign course.

In order to explore further the possibility of a cutaneous form of polyarteritis nodosa, all the case-notes of patients suffering from the disease at St Bartholomew's Hospital since 1949 have been analysed. Further, every patient of this series who had skin lesions has been personally examined and followed up subsequently.

PATIENTS

There were 54 men and 48 women, a total of 102 cases. In 37 the diagnosis was confirmed histologically, 19 at post-mortem (12 men and 7 women) and 18 by biopsy (11 men and 7 women). Eighteen patients (9 men and 9 women) had skin lesions, and biopsies were performed in all (Table I). One woman had eczema, 4 patients (3 women and one man) showed the histological changes of allergic vasculitis (leucocytoclastic angiitis), and 13 showed lesions of polyarteritis nodosa.

TABLE I.—*Types of Cutaneous Lesion*

Type	Male	Female	Total
Eczema		1	1
Allergic vasculitis	1	3	4
Polyarteritis nodosa			
Mid-dermis	1		1
Sub-cutis	7	5	12

Allergic vasculitis is the commonest type of vasculitis to affect the skin. The inflammation affects the capillaries and, to a lesser extent, the smallest arterioles and venules in the superficial dermis. The vasculitis is usually fibrinoid and the infiltrate leucocytoclastic. These features easily distinguish it from polyarteritis nodosa. The cutaneous features are also easily distinguishable, the lesions being superficial and polymorphic as opposed to the monomorphic deep dermal nodules of polyarteritis nodosa. Finally, this type of vasculitis may accompany a number of other collagen-vascular diseases, such as systemic lupus erythematosus, rheumatoid arthritis, dermatomyositis and systemic sclerosis. It is not surprising, therefore, that these 4 patients differed in no way from the cases without skin lesions. Two of the patients have died, histological evidence of widespread polyarteritis nodosa being found at post-mortem. In the 2 surviving patients the disease is manifested mainly in the lungs of one and in the kidneys of the other.

In the remaining 13 patients, microscopic examination of the cutaneous lesions showed the histological changes of polyarteritis. In 12 cases the lesions were in the subcutis, but in one they were scattered throughout the dermis.

This latter case was unique. He was a man of 44 who died of acute oliguric nephritis 5 weeks after the onset of his disease. Renal biopsy showed the lesions of polyarteritis nodosa. Four days before he died a widespread papulo-erythematous eruption developed of the type usually rather loosely referred to as toxic erythema. Biopsy showed a widespread necrotizing vasculitis throughout the dermis, a picture which was quite unlike either that of allergic vasculitis or of the lesions of cutaneous polyarteritis nodosa to be described. The rapid course of the disease recalls Kussmaul and Maier's (1866) original patients and clearly separates the case from the other 12. These form a remarkably cohesive clinical and histological picture, the only tissues to be affected being the skin, muscles and peripheral nerves (Table II), and a firm diagnosis could be made in every case. In order to emphasize this, 3 other cases seen at another hospital are included in the following description, making a total of 15 cases.

TABLE II.—*Clinical Findings*

System	Male	Female	Total
Skin	9	6	15
Muscle	4	3	7
Nerves (peripheral)	5	1	6

Cutaneous Manifestations

There were 9 men and 6 women, the age of onset varying from 17 to 61 (Table III). The age and sex had little bearing on the severity or course of the disease, although, on the whole, the earlier the onset the more acute and widespread were the cutaneous lesions. These consisted of 4 components, nodules, livedo, ulceration and incipient gangrene in that order of frequency (Table IV). The nodules tended to appear in crops varying in number from a single lesion to 6 or more at a time (Fig. 1). The commonest sites were the foot, ankle and lower leg, which were involved in all 15 cases, but they also occurred on the thigh (4 cases), hands and arms (5 cases), trunk (3 cases) and head and face (4 cases). There were no mucosal lesions. Two cases, one man and one woman, had recurrent nodulation all over the skin. The nodules were always tender, usually painful, and sometimes caused such exquisite pain as to render sleep impossible. They varied in size from 0·5 to 2 or 3 cm in diameter, the smaller lesions lasting from a day or two to a week, whereas the larger ones might last 2 or 3 weeks.

TABLE III.—*Age and Sex Incidence*

Age in years	Men	Women
10–19		1
20–29	1	1
30–39	3	3
40–49	3	
50–59		1
60–69	2	
Total	9	6

TABLE IV.—*Morphology of Cutaneous Lesion*

Type	Men	Women	Total
Nodulation	9	6	15
Livedo	4	6	10
Ulceration	2	1	3
Gangrene	2	0	2

Livedo occurred in 10 patients (6 women and 4 men). In 6 it was confined to the feet and lower legs (Fig. 2), but it also affected the hands and arms in 3 and the trunk in one. It tended to be confined to the area of maximal nodulation and, whereas nodulation frequently appeared in sites unaffected by livedo, the reverse did not occur.

Ulceration occurred in 3 cases (one woman and 2 men). In 2 it was limited to the area of nodulation and livedo, occurring only on one ankle in the woman (Fig. 3), but affecting the shins as well in one of the men. It was deep, persistent and painful in the former, but superficial and self-healing in the latter. In the third patient, in whom nodulation and livedo were universal, ulceration also occurred intermittently in all areas. It was evanescent and superficial in all areas except the nose, where it caused partial destruction of both nostrils.

Incipient gangrene occurred in 2 men, affecting the toes in both. One toe was affected in one case (Fig. 2) and all 10 in the other. They were cold, grossly cyanosed and extremely painful. Complete recovery occurred in both patients without loss of tissue.

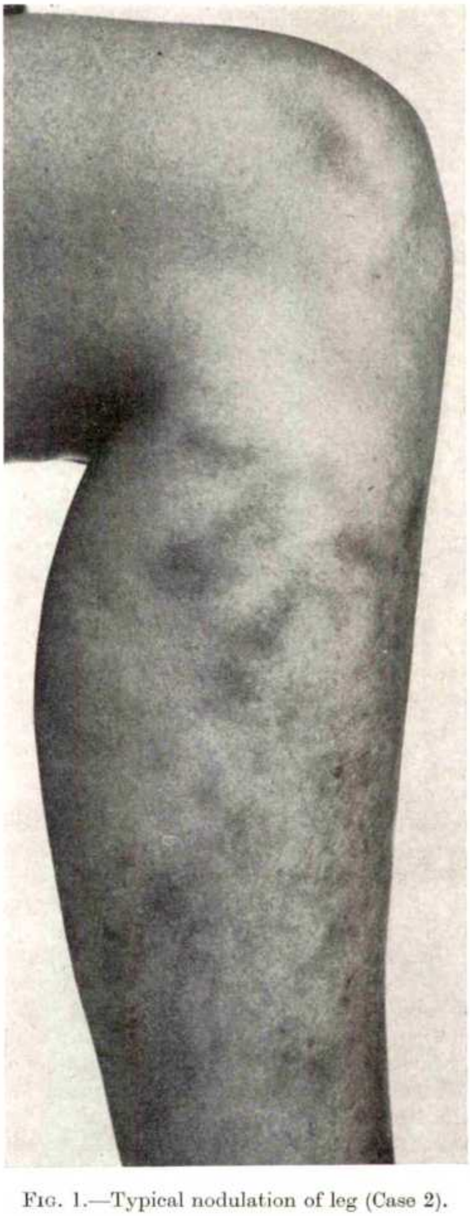

FIG. 1.—Typical nodulation of leg (Case 2).

Microscopical Lesion

Biopsy of a nodule showed a necrotizing arteritis involving the small arteries lying in the superficial sub-cutis, directly below the dermis (Fig. 4). Usually a single vessel was maximally involved, although a number of arterioles in its vicinity might also show some endothelial swelling, perivascular oedema and inflammatory cell infiltration. The severity of the arteritis varied widely. In some cases there was complete destruction of the vessel wall, with replacement by brightly eosinophilic fibrinoid material, often infiltrated by polymorphonuclear leucocytes. Leucocytoclasis was sometimes present, but seldom pronounced. In these cases the necrosis usually affected the whole circumference of the vessel, but was occasionally segmental. On the other hand, the vessel wall might show only fibrinoid deposits in the intimal region, with little leucocytic reaction. One of the most striking features was the localization of the inflammation to the immediate vicinity of the affected vessel and the absence of infarction in the neighbouring tissues. This accorded exactly with the main clinical finding of nodulation without surrounding inflammation.

There was little correlation between the severity of the microscopic lesion and that either of the individual cutaneous lesion or the disease as a whole.

Myositis

Myositis occurred in 7 patients. This was usually confined to the calves, causing aching pain, sometimes of extreme severity, together with stiffness. The latter was frequently described by the patients as an inability to place the heel on the ground when walking. In 2, however, both women, the myositis was widespread. In one of these cases myositis of the ankles and calves had been severe during the first episode of the disease, associated with equally painful nodulation in the same areas. Two years after all signs of the condition had disappeared, it recurred severely in the back and limbs, associated with an E.S.R. of 50 mm/1 hour, but without any cutaneous involvement. It persisted for 8 months, but finally cleared completely without any sequelae. In the second patient, in whom nodulation was widespread for many years, intermittent attacks of myositis in the legs, back, shoulders and neck also occurred. Muscle biopsies were performed in 5 of these cases, in all of which the microscopic lesions of polyarteritis nodosa were found.

Neuritis

Peripheral neuritis occurred in 6 patients (one woman and 5 men), with varying degrees of severity. One man complained of numbness of both feet, but no abnormal physical signs were found. In all the others there were sensory disturbances, pain, weakness and absent reflexes. The feet and lower legs were involved in all, unilateral in one and bilateral in the rest. The hands and forearms were involved in 2, one man and one woman. In the former both feet and lower legs had been involved at the outset, but recovery occurred in about 18 months. Six months later, however, an acute relapse occurred, with incipient gangrene of the toes and peripheral neuritis of both hands and wrists. Again complete recovery occurred. The woman was the most severe case of all, peripheral neuritis in all 4 limbs causing permanent unilateral foot drop and wasting and contractures of the fingers and hands.

One patient, a woman in whom nodulation and livedo began at the age of 18, suffered from superior vena cava obstruction 6 years later. At operation a fibro-inflammatory intra-vascular stricture was found at the junction of the azygos vein and the superior vena cava. Histological examination showed non-specific inflammation, but no evidence of polyarteritis nodosa. There have been no further symptoms over the subsequent 4 years, other than continuing cutaneous nodulation.

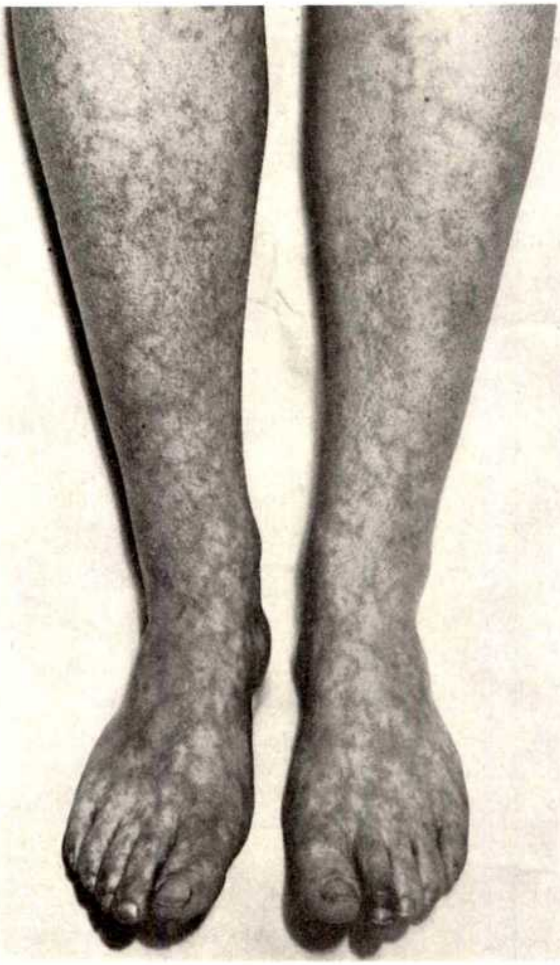

FIG. 2.—Marked livedo reticularis, with incipient gangrene of left second toe (Case 12).

Investigation

The few abnormal results found after extensive investigations are shown in Table V.

TABLE V.—*Investigations*

	Male	Female	Total
Fever	1	1	2
Raised E.S.R.	4	4	8
Anaemia	—	1	1
Leucocytosis	1	1	2
Rose–Waaler	—	1	1

Two patients, one man and one woman, were pyrexial and had a polymorphonuclear leucocytosis of 22,000 of a total W.B.C. count of 29,000, and 49,840 of a total of 56,000 respectively. The woman was the only patient found to be anaemic (Hb 48%). Both these patients had raised E.S.R.s and so, between them, they were responsible for the majority of the abnormal findings. The raised E.S.R.s varied between 31 and 80 mm

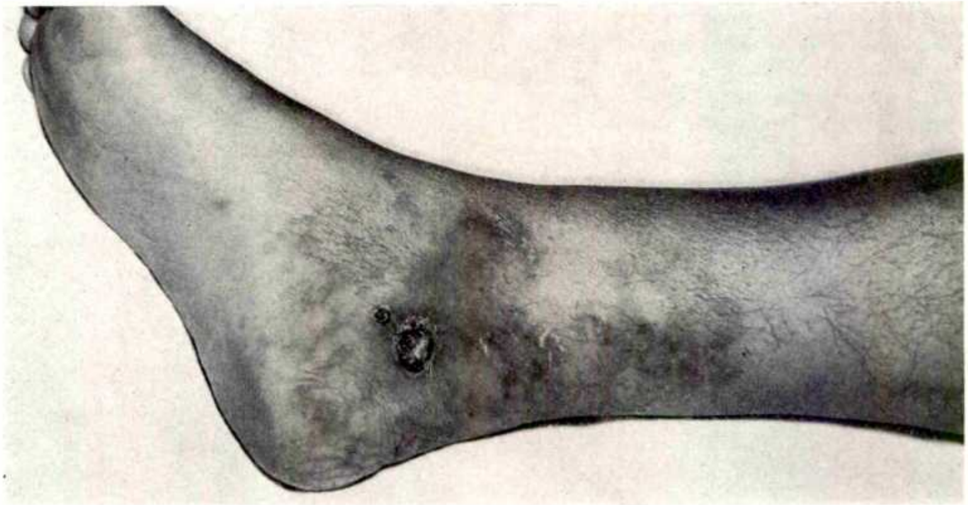

FIG. 3.—Nodulation and ulceration (Case 13).

TABLE VI.—*Course*

Case no.	Age at onset (years)	Sex	Follow-up (years)	Present condition
1	18	F	9	Nodulation
2	26	F	23	Peripheral nerve palsy; otherwise symptom-free (2 years)
3	28	M	3	Nodulation. Ulceration
4	31	F	18	Symptom-free (2 years)
5	32	F	15	Symptom-free (11 years)
6	33	M	17	Died (rheumatic heart disease)
7	33	M	14	Died (coronary thrombosis)
8	38	M	9	Nodulation
9	38	F	2	Died (suicide)
10	43	M	7	Nodulation
11	44	M	13	Symptom-free (6 years)
12	51	M	5	Nodulation. Ulceration
13	52	F	11	Nodulation
14	60	M	13	Symptom-free (2 years)
15	61	M	14	Symptom-free (6 years)

Figures in parenthesis indicate number of years of freedom from symptoms.

in the hour. None of these findings persisted for longer than the initial acute stage of the disease, although the E.S.R. became abnormal again if relapse occurred.

Course

The natural history of the condition is shown in Table VI. No patient has died of the disease, and the tendency has been for the condition slowly to fade away. The myositis and neuritis clear up first, usually lasting only a matter of months. The nodulation, however, may last for years, albeit with gradually decreasing frequency and severity. After a number of years treatment is no longer necessary and, in most of these cases, recent follow-up has been conducted by post.

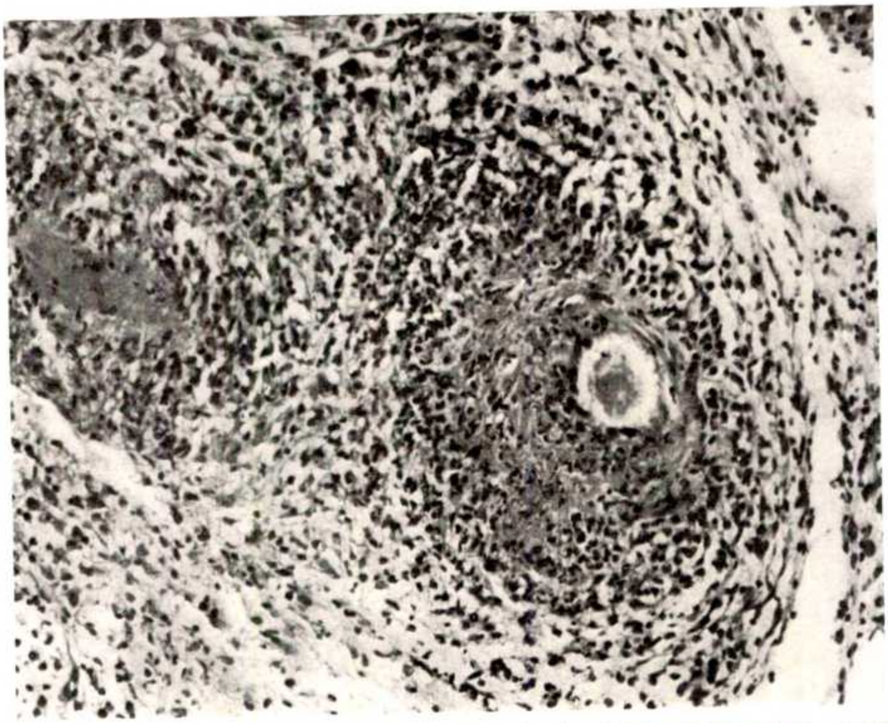

FIG. 4.—Necrotizing arteritis at junction of dermis and subcutis (Case 5) (H. and E. × 150).

Five patients, 2 women and 3 men, had relapses after apparent recovery. One of the women (Case 5) had a relapse 2 years after the initial phase had cleared. This relapse (already referred to) consisted entirely of a widespread myositis accompanied by an E.S.R. of up to 50 mm/1 hour. The second woman (Case 2) suffered 2 relapses 4 and 9 years after the initial episode. Both affected the skin alone and were associated with a raised E.S.R. and a polymorphonuclear leucocytosis, but no fever. They each lasted a number of months, the first one following tonsillitis treated with penicillin.

One of the men (Case 6) had 2 minor cutaneous relapses, both following trauma to the legs. Another (Case 12) is at present suffering his second relapse, the disease being confined to the skin of the feet and legs. These relapses have occurred 4 and 5 years after the initial recovery. The last man (Case 14) relapsed 6 months after the initial episode, with a sudden bilateral wrist- and foot-drop and painful incipient gangrene of the toes. He made a full recovery in a few months and has had no further trouble.

Post-mortem examinations were performed on all 3 patients who died. Two were coroner's post-mortems, and no mention of polyarteritis nodosa was made in either, the causes of death being coronary thrombosis in one and an overdose of hypnotics in the other. The third post-mortem was performed in hospital and was followed by extensive histological examination. The only evidence of polyarteritis nodosa was found in the skin of the legs, and this consisted of healed lesions.

Aetiology

The cause of the disease in these cases was as ill-defined and problematical as in the systemic form. In only 3 patients was there a possibly relevant preceding or accompanying history. In one man the disease began 2 months after a 9 months course of streptomycin for pulmonary tuberculosis, from which he made a complete recovery. The polyarteritis nodosa was mild and confined to the skin and muscles of the lower legs (Case 7).

The disease in the second patient (Case 2) was entirely cutaneous for the first year, but then became very severe, with fever, leucocytosis, high E.S.R. and neuropathy in all 4 limbs, following tonsillitis treated with sulphonamide. A similar but less severe exacerbation followed tonsillitis treated with pencillin 11 years later.

In the third case (Case 3) tonsillitis treated with a variety of antibiotics resulted in a severe universal cutaneous eruption and arthritis. The ASO titre was 330. The patient made a complete recovery on prednisolone. No biopsy was performed. Three years later exactly the same thing happened, but this time steroids did not assist, and the ASO titre was found to be 3300. He made a complete recovery on penicillin. Skin biopsy showed leucocytoclastic angiitis of the superficial dermal vessels (allergic vasculitis), but no involvement of the subcutaneous arterioles. Such a histological picture is not uncommon in severe drug eruptions. Following this the prednisolone was continued in a dose of 10 mg a day for one year. One month after discontinuing the steroids his present condition of universal livedo, nodulation and patchy, superficial ulceration began. Histological examination at this time showed the classical picture of cutaneous polyarteritis nodosa.

During the first illness he also suffered from acute nephritis. However, 5 years later and one year after the onset of the cutaneous polyarteritis nodosa, extensive renal function tests were completely normal.

Treatment

These patients have been treated with steroids and analgesics. The dose of the former has rarely been more than 20 mg of prednisolone a day, and usually less. The aim has been to control the acute exacerbation and relieve pain, rather than to suppress all manifestation of the disease. As the condition has become less severe, it has been possible to discontinue treatment altogether in most patients and, at the present time, only 2 patients (Cases 1 and 3) are receiving treatment (prednisolone 5–15 mg daily).

DISCUSSION

Analysis of these cases leaves little doubt that there is such an entity as cutaneous polyarteritis nodosa. Further, the condition is easily recognizable both clinically and histologically. It is essential, however, to recognize the criteria on which the diagnosis of this condition is based. For instance, Ketron and Bernstein's (1939) case, from which so much misunderstanding has stemmed, was a straightforward example of acute systemic polyarteritis, ending in death in 17 weeks, with histological evidence of involvement of the heart, kidneys, gut, skin and pulmonary artery. The fact that there was also universal livedo with ulceration does not turn it into a case of cutaneous polyarteritis nodosa and it is more than significant that nodulation, the hall-mark of the cutaneous lesion in the latter condition, was absent. The case was not unlike that of acute, mainly renal, polyarteritis nodosa already described in this paper. Involvement of the skin does not necessarily mean that the case is one of cutaneous (benign) polyarteritis nodosa and therefore has a good prognosis.

On the other hand, it is striking that, of 102 cases in the present series, only 13 had histological evidence of specific cutaneous lesions and 12 of these were clinically of the cutaneous (benign) type. Thus a positive cutaneous biopsy is strongly suggestive of the benign type and, if the lesion is in the subcutis and the feature biopsied was a nodule, the findings are virtually pathognomonic.

Further investigation and observation of these cases may show that occasionally systemic lesions develop. At present, however, there is no evidence of this.

I wish to thank the physicians of St Bartholomew's Hospital and Dr P. Montgomery for permission to examine their cases; Dr Alfred Stansfeld for both the histological reports and generous assistance with all pathological aspects; and the Department of Medical Illustration, St Bartholomew's Hospital, for the photographs.

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CYTOLOGY OF THE MYCOSIS FUNGOIDES AND THE SÉZARY SYNDROME

CORRELATION BETWEEN LIGHT AND ELECTRON MICROSCOPY

J. GOMEZ ORBANEJA, E. SANCHEZ YUS, L. DIAZ-FLORES AND
P. SIMON HUARTE

Summary.—Nine skin biopsies and 2 lymph node biopsies obtained from 6 patients with mycosis fungoides and one with Sezary syndrome were studied. Touch smears were also made. Two main cells were found in the infiltrates: mycosis fungoides cells (MC) and histiocytes. The size of the former varied between 10 and 30 μm and consequently the smaller ones were similar to lymphocytes as viewed by light microscopy. With the electron microscope they had a similar structure to the cells described by Lutzner and other writers, with slight variations according to the size of the cell. Either of the 2 cells could undergo malignant transformation, the other cell persisting as a benign associated feature. Thus mycosis fungoides can become malignant either as mycosis fungoides by malignant transformation of the MC or as reticulum-cell lymphoma by malignant transformation of histiocytes. Neither histological nor cytological differences can be found between the MF and the Sezary syndrome.

THE lack of an easily identified cell in all phases of mycosis fungoides (MF), as well as the variable histopathology at different stages of the disease, have been the main reasons for the considerable confusion concerning its pathology. The primitive mycosis fungoides cell described by Nanta (1936), with voluminous cytoplasm and clear oval nucleus with a large nucleolus, was not accepted as specific even by his own contemporaries (Pautrier, *et al.* 1937; Zoon, 1937). In fact, such a cell could represent either a hyperplastic reticulum cell or, possibly, a malignant reticulum cell (Sánchez Yus, 1970). Recently Allen (1967), Lever (1964), Pinkus and Mehregan (1969) and Sánchez Yus (1970) have described a mononuclear cell with a hyperchromatic nucleus and sparse basophilic cytoplasm, to which has been attributed some degree of specificity as the characteristic cell of MF. But this cell can be demonstrated only with difficulty in the "premycosis" stages, and may also be difficult to find in infiltrated plaques and tumours. Recently electron microscopic studies have demonstrated the peculiar structure of this cell both in the Sézary syndrome (Lutzner and Jordan, 1968; Ebner *et al.*, 1970; Bourlond *et al.*, 1970) and in the skin and lymph nodes (Lutzner, 1968; Brownlee and Murad, 1970) of patients with MF. This is the cell which is here referred to as the mycosis fungoides cell (MC).

In the present study we propose a unitary concept for MF and the Sézary syndrome. The existence in the infiltrate of 2 basic cells (the MC and the histio-

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cyte), each possessing a potentiality for becoming malignant, appears to be responsible for the varying histological features of the infiltrated plaques and tumours of MF.

MATERIALS AND METHODS

Nine biopsies of skin and 2 of lymph nodes, from 7 MF patients were examined both by light and electron microscopy.

The bone marrow and peripheral blood smears of the 7 patients and the touch smears of 4 skin biopsies and a lymph node were also studied by light microscopy.

The fragments for the electron microscope were fixed in glutaraldehyde buffered by sodium cacodylate for 2 hours, post-fixed for 2 hours in osmium tetroxide, and embedded in vestopal. The sections obtained with an ultramicrotome LKB were counterstained with uranyl acetate and lead citrate (according to Reynolds) and examined with a Philips EM 200 microscope.

The blood films and touch smears were stained with May–Grünwald–Giemsa. All touch smears, as well as the peripheral blood of one of the patients that showed abnormal cells, were stained with PAS.

The following were studied with the electron microscope as control cases: 7 lymph nodes from 3 cases of Hodgkin's disease; 2 cases of tuberculous lymphadenitis; one lymphocytic lymphoma and one reticulum cell lymphoma. For comparison, various inflammatory skin processes were also studied: a case of leishmaniasis and the inflammatory reactions to various types of squamous cell carcinoma, keratoacanthoma and malignant melanoma.

RESULTS

In all biopsies using the *electron microscope*, both skin and lymph nodes (Table I) showed the presence of large numbers of peculiar cells which had the basic characteristics of the cells described by Lutzner (1968) and Lutzner and Jordan (1968). The nuclei of the cells showed deep and tortuous foldings of the membrane, and abundant peripherally distributed chromatin. Usually a single nucleolus of medium density was found. The cytoplasm was sparse, with numerous free ribosomes, some mitochondria, a rarely detectable Golgi apparatus, and an almost total lack of endoplasmic reticulum. They lacked lysosomes, and there was no evidence of phagocytosis.

These unusual cells were found in the epidermis, either as single cells or forming small groups. In the dermis and in the lymph nodes they were dispersed between other mesenchymal cells. In the different biopsies there was considerable variation in (1) size and various characteristics of the MC; (2) the percentage number of the cells; and (3) the nature of the accompanying cells.

The MC were small at times (Fig. 2A) with a nucleus approximately 7 μm in diameter; the invagination of the nuclear membrane was prominent, so that often the nucleus appeared to be divided into separate parts joined by thin bridges; at times they resembled bi- or trinucleated cells, according to the plane of the section. The cytoplasm was sparse and almost completely lacking in organelles. Mitotic figures were not seen. In other specimens the MC were larger (Fig. 2C) with nuclei up to 22 μm, usually eccentrically situated in a more abundant cytoplasm containing many mitochondria. The nuclear membranes were less markedly indented, but atypical mitoses were frequent. In other fields, or in different biopsies, the intermediate grades between these 2 different types of cell could be

TABLE I.—*Clinical, Histological, Cytological and Ultrastructural Data*

Case	Sex and age (in years)	Duration (years)	Clinical data	Blood changes	Type of lesion	Histology	Cytology	Ultrastructure
1	F31	11	Variably infiltrated disseminated plaques and tumours	No	(a) Slightly infiltrated plaque	Acanthosis. Lymphocytes, histiocytes. No exocytosis	—	Histiocytes, lymphocytes and small MC
					(b) Non-ulcerated tumour	Acanthosis. Histiocytes. Some Pautrier microabscesses	Histiocytes and lymphocytoid cells PAS-negative	Histiocytes, small MC
					(c) Non-ulcerated tumour	Epidermal atrophy. Histiocytes, lymphocytes eosinophils, Pautrier microabscesses in follicles	Same	Same
2	M53	3	Scaly exudative disseminated erythematous plaques	No	Premycosis	Psoriasiform epiddermis. Lymphocytes, histiocytes, MC. Exocytosis	—	Histiocytes, some lymphocytes, small MC
3	F46	24	Generalized psoriasiform plaques, slightly infiltrated. No tumours. Slightly enlarged lymph nodes. Pruritus. Alopecia	No	Premycosis	Same, plus some eosinophils and medium MC	—	Histiocytes, lymphocytes, variably sized MC
4*	M59	1	Numerous eczematoid plaques and ulcerated tumours. Enlarged lymph nodes. Alopecia	No	(a) Ulcerated tumour	Reticulum-cell lymphoma; some lymphocytes	Malignant reticulum cells, some small PAS-negative MC	Malignant reticulum cells, small MC
					(b) Inguinal lymph node	Band of lymphocytes with eosinophils and plasma cells	Lymphoid cells (20%PAS+), eosinophils. Reticulum cells	Small MC, reticulum cells, eosinophils

5	M41	3	Infiltrated round erythematous plaques with central necrosis. Ulcerated tumours. Slightly enlarged lymph nodes	No		Infiltrated plaque	Predominance of large MC; mitosis; intense exocytosis	Large MC; many mitoses	Large MC; many mitoses
6	M69	2	Generalized erythroderma. Pigmentation. Palmoplantar keratoderma. Hepatomegala. Enlarged lymph nodes	10,000 leukocytes. 13% Sézary cells	(a)	Infiltrated plaque	Acanthosis. Lymphocytes, histiocytes, eosinophils, plasma cells. Exocytosis	—	Small MC, histiocytes
					(b)	Inguinal lymph node	Areas similar to 4(b)	—	Same as 4(b)
7	M34	4	Large infiltrated eyrthematous plaques. Parapsoriasis " en plaques " type. No enlarged lymph nodes	No		Infiltrated plaque	Epidermal atrophy. Medium MC, histiocytes, eosinophils Pautrier microabscesses	—	Medium MC, histiocytes, eosinophils

* Large number of MC and Pautrier microabscesses in the eczematoid plaques.

seen, but a manifest similarity of their basic morphology was seen in spite of these variations.

The small MC type was particularly predominant in biopsies: 1b and 1c (plaque and tumours), 2 (premycosis) (Fig. 2A), 4b (specific adenopathy) (Fig. 3D), 6a and 6b (skin and lymph node of a Sézary syndrome) (Fig. 3B, C). They were accompanied by numerous normal histiocytes and in biopsy 1c by some eosinophils. The scarcity of lymphocytes was noteworthy since, under the light microscope, they seemed to be very abundant (Fig. 1A). In the tumour of Case 1 (b and c) there were zones formed almost exclusively by MC (Fig. 1A) and others with an almost total predominance of histiocytes (Fig. 1B). These histiocytes appeared

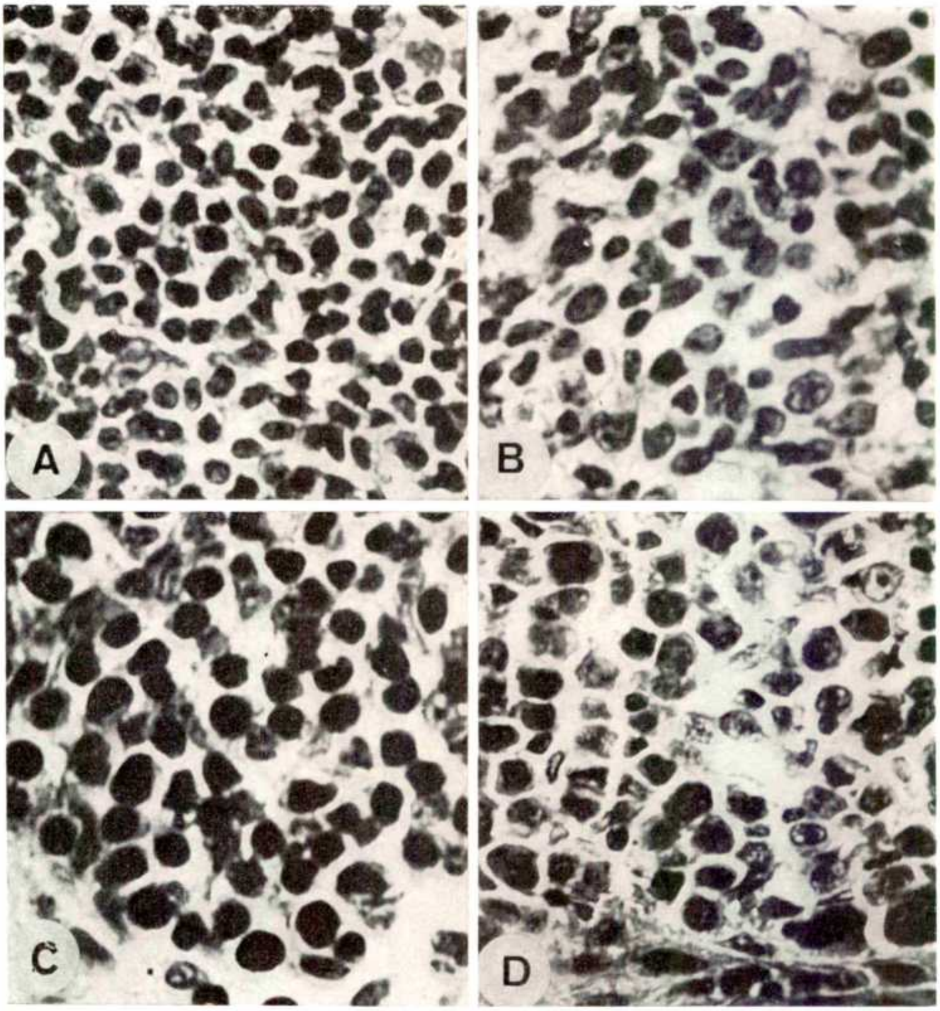

FIG. 1.—Different cell types of MF under the light microscope. A, Small MC cannot be distinguished from lymphocytes (Biopsy 1b). B, Histiocytes in another field of the same biopsy. C, Large MC colonizing the epidermis (Biopsy 5). D, Pleomorphic reticulum-cell sarcoma (Biopsy 4a). (H. and E. × 400)

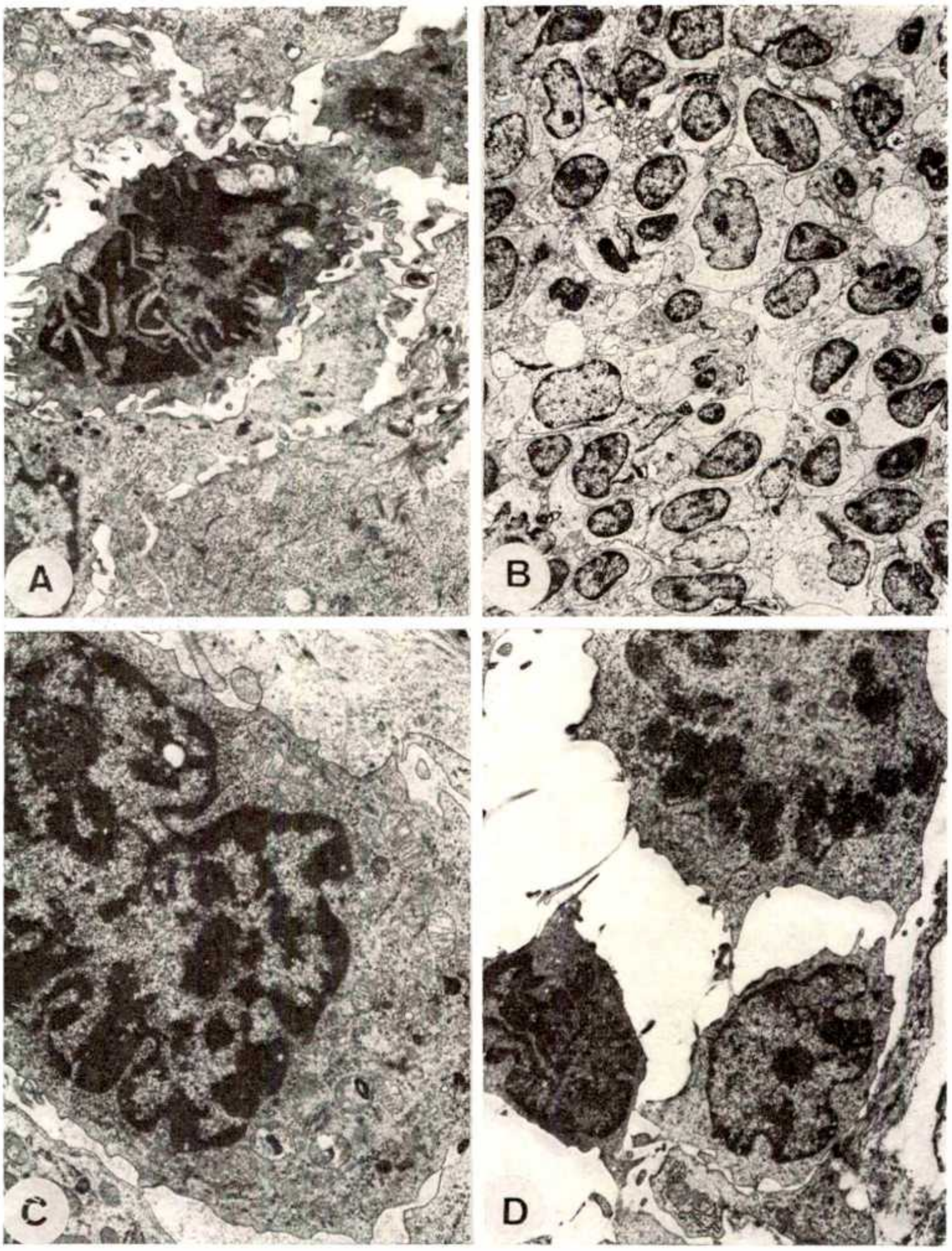

FIG. 2.—The same cell types as in Fig. 1 under the electron microscope. A, Small MC in the epidermis, with characteristic nucleus and sparse cytoplasm (× 10,000). B, Histiocytic zone equivalent to Fig. 1B (× 3000). C, Large MC from the biopsy of Fig. 1C (× 11,500). D, Two malignant reticulum cells (one in mitosis) and one small MC from the tumour of Fig. 1D (× 8100).

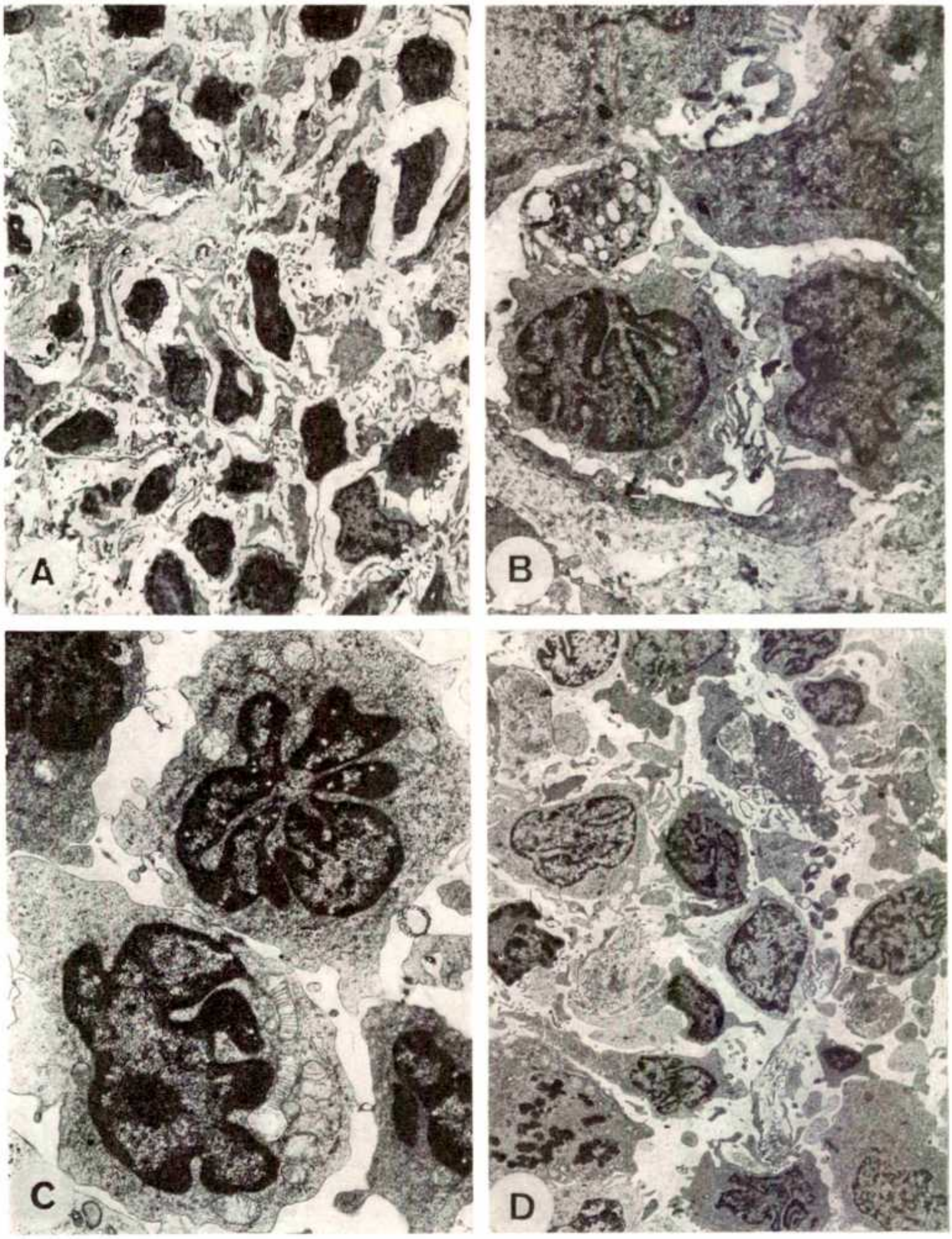

FIG. 3.—A, Small MC with abundant fine, large cytoplasmic extensions (Biopsy 1c) (× 3000). B, Two MC penetrating the epidermis, Sézary syndrome (Biopsy 6a) (× 7500). C, MC in lymph node of the same patient (Biopsy 6b) (× 9500). D, Pleomorphic aspect of a MF lymph node with MC and reticulum cells (one mitosis) (Biopsy 4b) (× 3000).

hyperplastic but not malignant (Fig. 1B, 2B). The MC at times were peculiar (as in Case 2) in possessing numerous fine, large cytoplasmic extensions (Fig. 3A). In the 2 lymph nodes the accompanying cells were mainly reticulum cells and eosinophils (Fig. 3D). In the skin tumour of Case 4, composed of pleomorphic reticulum cells by light microscopy (Fig. 1D), the MC (small size) were merely an associated feature (Fig. 2D). The reticulum cells had large and clear nuclei, with large irregular nucleoli and extensive cytoplasm, rich in organelles. Atypical mitoses were frequent. The large MC predominated in the biopsy of Case 5, where its marked epitheliotropism was noteworthy (Fig. 1C, 2C), with cells invading the hair follicles and the sweat glands as well as the epidermis. The dermal infiltrate was largely monomorphous, with sparse histiocytes. Mitoses were numerous. The biopsy of Case 3 was characterized by the MC showing great variation in size, while in Case 7, cells of an intermediate type predominated. In none of the controls studied with the electron microscope was the MC, as described here, found.

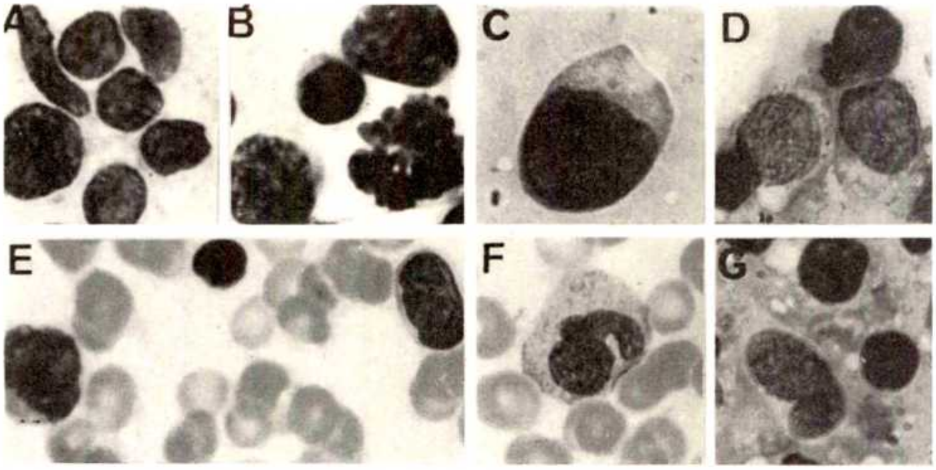

FIG. 4.—Skin and peripheral blood smears (May–Grünwald–Giemsa, × 500). A, Small MC and one of larger size MC (Biopsy 1b). B, Three large MC, one in mitosis, and one lymphocyte (Biopsy 5). C, Large MC from the same case. D, Malignant reticulum cells and 2 lymphoid cells (Biopsy 4a). E, One Sézary cell (left), one lymphocyte (centre) and one monocyte (right). Peripheral blood, case 6: F, one monocyte. Peripheral blood from the same case. G, One histiocyte and 2 small MC (Biopsy 1b).

The smears showed the same differences of size in the MC (Fig. 4A, B, C) as with the electron microscope. It was easier to distinguish the smaller MC from lymphocytes in smears than in the sections, because the nuclear indentations were more apparent in the smears. The MC were PAS-negative in smears, except for 20% of cells obtained from the lymph node in Case 4; this positive staining could be removed by diastase digestion.

In one of the patients (Case 6) with an erythrodermic clinical form, 13% of the leucocytes in the peripheral blood were abnormal large cells with convoluted nuclei and a rather sparse, basophilic, PAS-negative cytoplasm (Fig. 4E). These cells were not found in the bone marrow.

DISCUSSION

Two initial conclusions can be drawn from the results of the present study and those previously mentioned: (1) In MF there exists a peculiar cell with defined ultrastructural characteristics; (2) there is no morphological difference between the MC and the so-called Sézary cell. The slight differences (greater nuclear irregularity in the Sézary cell) noted by Lutzner and Jordan (1968) can be discounted in view of later studies. This supports the views of Clendenning *et al.* (1964) and Allen (1967) that the Sézary syndrome is a form of MF in which the abnormal cells overflow into the blood.

This study also contributes other findings, relevant to the problems of MF.

First, we are able to confirm the observations of Lutzner (1968) that the MC varies greatly in size. A MC of 10 μm or less is practically indistinguishable by light microscopy from normal lymphocytes. We have shown that most of the " lymphocytes " are small MC on electron microscopy. Thus their presence can pass unnoticed, in both the infiltrate of a " premycosis " and in apparently non-specific adenopathies, as well as in tumours evolving towards other lymphomata (Biopsies 1a, 2, 4a, 6b). This is largely due to the fact that the diagnosis of MF in skin biopsies by light microscopy is based on secondary features such as the superficiality and polymorphism of the infiltrate, the formation of Pautrier microabscesses and the epidermal reaction; but it is only when MC of medium or large size are found that the diagnosis becomes certain and the " secondary signs " become merely a confirmatory aid to the diagnosis.

The variability in size of the MC as well as the variability in cytoplasmic organelles can explain the differences observed between the Sézary cells from different cases, including one patient (Ebner *et al.*, 1970) with cells showing lymphocytic as well as monocytic features.

Secondly, we have observed that malignant transformation in the infiltrated plaques or tumours of MF can occur by malignant transformation of either the MC or the histiocyte, the other cell persisting as a benign associated feature. The small MC hardly ever go into mitosis, but the large MC are often seen in abnormal mitosis, and such cells are presumably malignant MC. The infiltrated plaques of a patient in which this cell type predominated (Case 5), enlarged rapidly and ulcerated easily before they formed tumours, but the histiocytes were found not to be altered. A contrasting picture was observed in Case 4 in which some small MC accompanied an exuberant proliferation of malignant reticulum cells. In the first case the malignant transformation does not appear to indicate transformation of MF into another malignant reticulosis; but in the second case it appears to do so. We want to point out that, as has been seen in other patients (Sánchez Yus, 1970) and in 2 in this series (Cases 1 and 4), the transformation into another lymphoma does not signify a total transformation of the disease, but rather that it may occur as an occasional and localized event, since other plaques and tumours studied years later may continue to show a MF histology (Case 1). Histological features of MF were also observed in a lymph node which had been biopsied at the same time (Case 4).

The existence in MF of 2 cell series, the MC and the histiocyte, co-existing but evolving separately toward malignancy, is an argument against a possibly histiocytic origin for the MC. In addition, the ultrastructural characteristics of the MC suggest it to be an anomalous lymphoid cell. Recently, Crossen *et al.*

(1971) have made cytogenetic studies in the Sézary syndrome and have found abnormal chromosomal changes in Sézary cells, but the cells responded in a similar way to lymphocytes to various stimuli. Nevertheless, in 2 of our cases, we have found some MC (Biopsies 1b, 1c and 2) with cytoplasmic extensions suggestive of a histiocytic cell. Moreover the almost constant PAS-negativity found in the smears is not in favour of their lymphocytic nature.

The specificity of the MC as observed under the electron microscope has been scarcely doubted up to the present. Lutzner (1968) and Lutzner and Jordan (1968) did not find this cell in other malignant lymphomata or in inflammatory skin processes. No similar cells were observed in the numerous illustrations in the ultrastructural atlas of the normal and pathological lymph nodes of Mori and Lennert (1969). Nor have we found it in our controls. Recently however, Lutzner *et al.* (1971) have published an extensive study on the subject in which the discovery of cells ultrastructurally similar to MC in a reticulum cell sarcoma and in 2 lichen planus biopsies is notable. Therefore more extensive investigations will be necessary to establish its specificity with greater confidence and thus define the true limits of mycosis fungoides.

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THE EPIDERMAL COMPONENT OF PITYRIASIS LICHENOIDES

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Summary.—The scaling papules of pityriasis lichenoides (PL) can at times simulate psoriasis clinically and histologically. In this study particular attention has been paid to the epidermal changes in PL, and 126 biopsy specimens from 94 patients were examined. Parakeratosis was found in 72%, invasion of the epidermis by mononuclear cells in 69%, and focal cell death in 57%. Skin surface biopsies from 8 patients and cryostat sections from 12 patients were examined histochemically. The parakeratotic scale and scattered individual keratinocytes within the epidermis demonstrated strong hydrolytic enzyme activity, and there was a diminution in the reactivity for mitochondrial enzymes within the PL papules. The labelling index was determined for 7 PL lesions after injection of tritiated thymidine and found to be 15·2%. In addition, slices of tissue from 8 PL papules were incubated with tritiated proline, and this manoeuvre also demonstrated the existence of dead and dying cells within the Malpighian layer. The suggestion is made that parakeratosis in PL is caused more by damage to the epidermis than by increased epidermopoiesis.

THE commonest type of lesion in pityriasis lichenoides (PL) is a scaling papule. Occasionally the scaling is sufficiently pronounced to give the condition a superficial clinical resemblance to psoriasis. Histologically, there may also be a similarity to psoriasis, as there is often pronounced parakeratosis. In psoriasis there is a high rate of epidermal turnover (Van Scott and Ekel, 1963; Weinstein and Van Scott, 1965), and it is often assumed that the high rate of epidermopoiesis and decreased transit time that result from this, cause the parakeratosis. PL is a complex disorder, and it seemed justified to dissect out the epidermal component for special study, so as to help in understanding the histogenesis of the lesions. A further aim of this investigation was to determine whether the parakeratotic scale was associated with a high rate of cell turnover and produced in the same way as in psoriasis.

PATIENTS AND METHODS

All the patients studied satisfied the criteria for a diagnosis of PL established by Marks *et al.* (1972).

Histological Studies

One hundred and twenty-six specimens from 94 patients were available for study. Formalin-fixed and paraffin-embedded sections stained with H. and E. were examined for the type and extent of epidermal damage in each case.

Autoradiographic Studies

One mm thick vertical sections of fresh tissue were prepared from the scaling papules of 8 patients with pityriasis lichenoides. These tissue slices were incubated in Eagle's

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minimal essential medium in plastic petri dishes at 37°C in an atmosphere of 95% air and 5% CO_2 for 4 hours. The medium contained tritiated proline (Radiochemicals, Amersham, specific activity 6·8 Ci/mmol) at a concentration of 2 μCi/ml. At the end of 4 hours the incubated tissue was fixed in 10% formol saline, dehydrated and paraffin-impregnated. Sections were dipped in nuclear emulsion (Ilford K2), using the method of Joftes and Warren (1955), and exposed for periods of 2 to 3 and 4 weeks. Slides were developed in Kodak D 19 developer and then stained with H. and E. and examined microscopically.

Eight scaling papular lesions from 7 patients with PL were injected intracutaneously with 10 μCi of tritiated thymidine (Radiochemicals, Amersham, specific activity 22 Ci/mmol) in 0·1 ml of sterile normal saline. Seven injected lesions were removed after 50 min to one hour. One injected lesion was biopsied 3 days later.

The biopsied lesions were fixed, and autoradiographs were prepared from them, as in the method described above for the tissue incubated *in vitro*. These were then examined microscopically; in the sections prepared from tissue exposed to the tritiated thymidine for 50 min to one hour, the number of labelled basal and suprabasal cell nuclei per 1000 basal cells was counted.

Histochemical Studies

(a) Scaling papules from 12 patients with PL were removed and snap frozen in liquid nitrogen. They were sectioned on a Bright's cryostat, and sections were examined for the following enzyme activities:

1. Non-specific esterase (indoxyl acetate method of Holt, 1954).
2. Acid phosphatase (Gomori lead phosphate method, modified by Holt, 1954).
3. NAD diaphorase
4. Succinic dehydrogenase } using the NTB method of Pearse, 1960.
5. Lactic dehydrogenase

(b) The scale from several papules of 8 patients with PL was sampled, using the skin surface biopsy method of Marks and Dawber (1971). The skin surface biopsies were obtained on cover slips and examined by the enzyme histochemical methods used for cryostat sections as described above. The resulting specimens were examined microscopically.

RESULTS

Histological and Histochemical

A striking feature of the histological appearance in all the specimens examined was the sharply focal distribution of the disturbance both in the dermis and epidermis. The main histological changes affecting the epidermis were:

Feature	Degree	Numbers		Percentage of total	
Parakeratosis	Slight	49	91	39	72
	Marked	42		33	
Focal cell death	Slight	30	72	24	57
	Marked	42		33	
Invasion by leukocytes	Slight	49	88	39	69
	Marked	39		30	
Eczema change	Slight	73	86	57	67
	Marked	13		10	

Seventy-two % showed parakeratosis (Fig. 1). Of the other 28% there was either frank necrosis of the epidermis or very little scale to see, presumably because of its separation and loss during preparation. Interspersed within the parakeratotic scales were small collections of mononuclear inflammatory cells, and invasion of the epidermis by such cells was seen in 69%. Another feature was the presence of swollen eosinophilic cells scattered in the Malpighian layer in 57% of cases (Fig. 2). These cells resembled the hyaline blocks found in the basal layer in lichen planus. They were interpreted as dead or dying cells, and were sometimes quite numerous. Apart from parakeratosis, invasion by inflammatory cells and focal cell death, spongiosis and slight irregular acanthosis were also commonly seen.

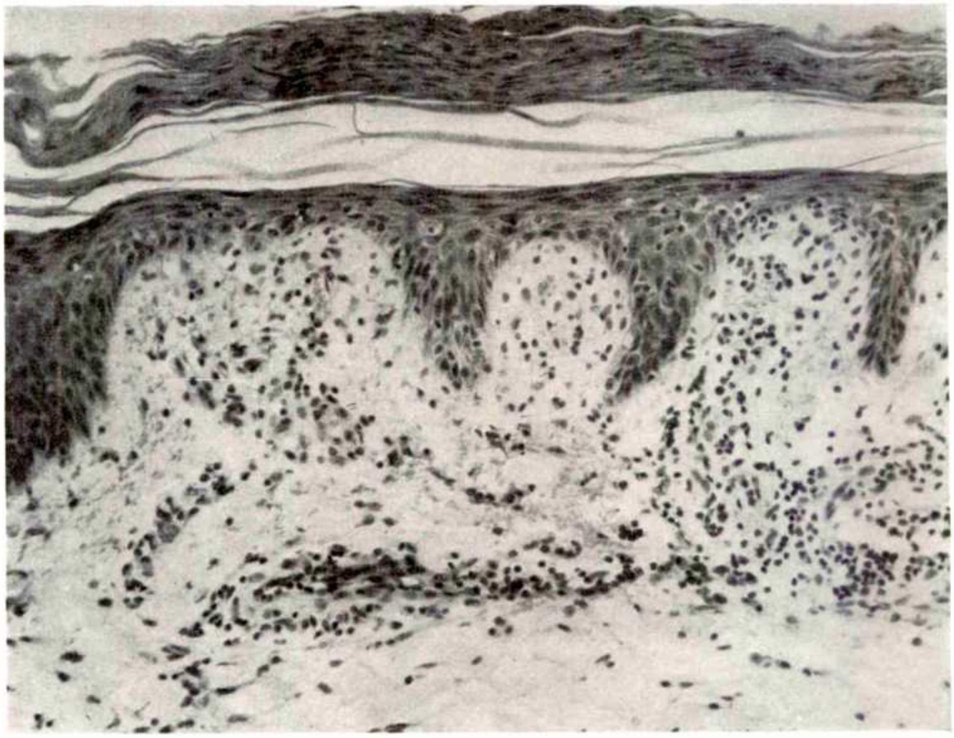

FIG. 1.—Detaching parakeratotic scale seen in biopsy from papule of PL. There is also oedema and a perivascular inflammatory cell infiltrate in the papillary and subpapillary zones (H. and E. × 45).

Enzyme Histochemical Results

Non-specific esterase and acid phosphatase reactions.—The epidermis of the scaling papule did not demonstrate the usual strong reactivity for these hydrolase activities in the granular layer. Instead, the surface scale showed intense reactivity, and there were individual keratinocytes within the Malpighian layer that showed strong activity for these hydrolytic enzymes. Skin surface biopsies from scaling PL papules demonstrated intense hydrolytic activity sharply localized to the scale from the lesion (Fig. 3).

Mitochondrial enzyme reactions.—The NAD diaphorase succinic dehydrogenase and lactic dehydrogenase reactions showed essentially the same pattern of reaction. There was an abrupt decrease in the deposition of formazan reaction product in the epidermis of the affected papule, and there seemed to be a rough correlation between the degree of inflammatory disturbance and the depression in the enzyme reactivity within the papule. Skin surface biopsies taken from the scaling papular lesions and tested for NAD diaphorase and lactic dehydrogenase activities did not demonstrate the striking perinuclear deposition of formazan granules seen in the parakeratotic scale from psoriasis.

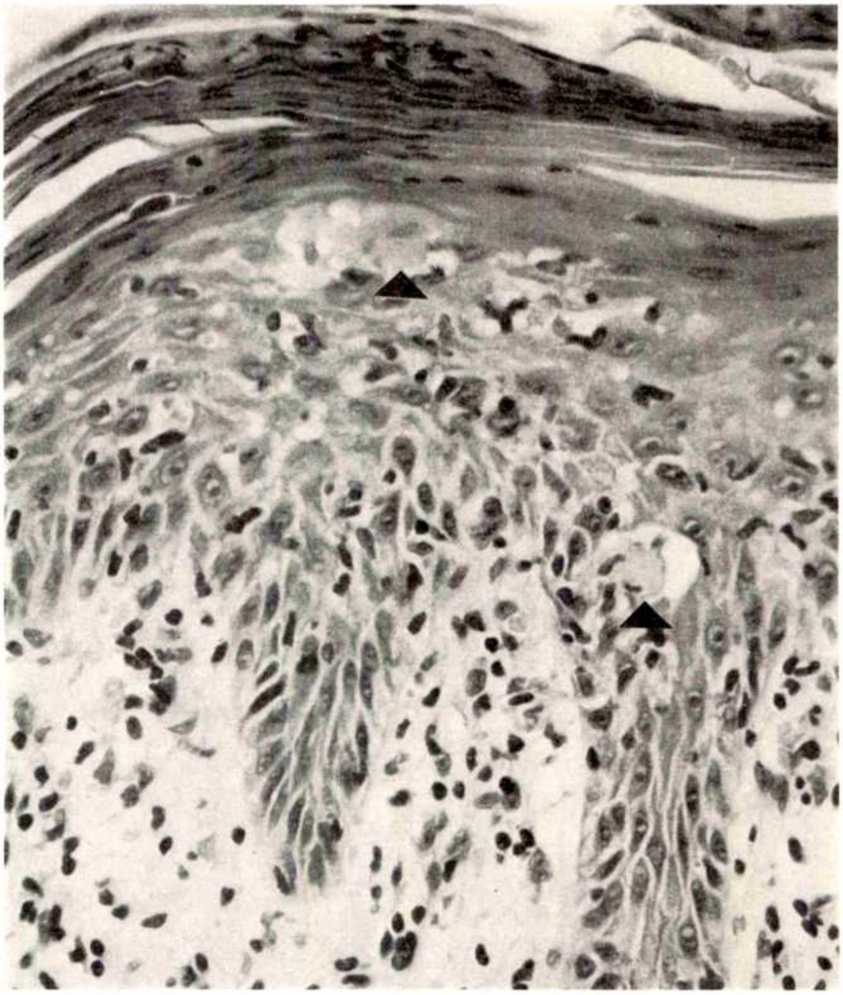

FIG. 2.—Area of epidermis in papule of PL. There are 2 swollen and pale epidermal cells in this photomicrograph (designated by triangles). In addition there is a parakeratotic scale, invasion of the epidermis by inflammatory cells, and epidermal oedema (H. and E. × 90).

Autoradiographic Results

The labelling indices for the 7 biopsies assessed, expressed as a percentage, were as follows: 8·7, 10·9, 6·2, 10·0, 19·0, 15·0, 7·2. After applying the correction advocated by Weinstein and Frost (1969) by multiplying by 1·4, the mean corrected labelling index is 15·2% (S.E. ± 2·2). There was some variation of the degree of nuclear labelling within the individual biopsies and, in very inflamed areas, there tended to be more labelled cells. In the biopsy removed after injection with tritiated thymidine 3 days previously, labelled cells had not reached the granular cell layer (Fig. 4). Those tissue sections incubated with tritiated proline and processed autoradiographically showed the highest concentration of silver grains over the basal cells, with a decreasing concentration towards the stratum corneum. In 5 of the specimens examined there were large eosinophilic cells interspersed within the epidermis that did not show silver grains over them (Fig. 5).

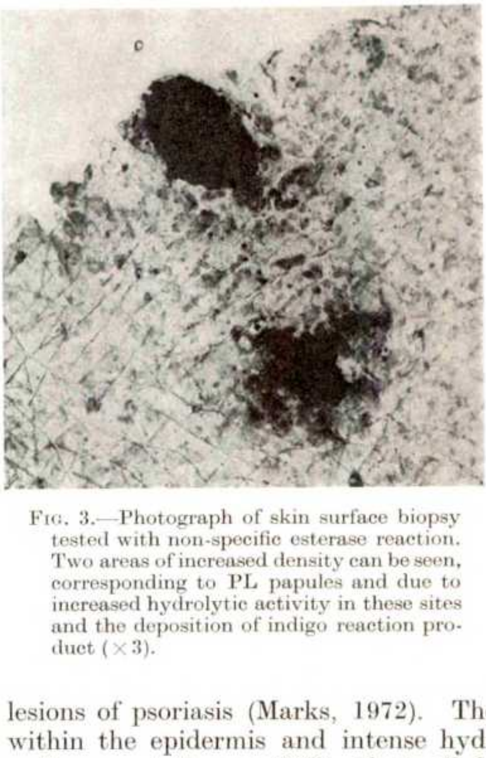

FIG. 3.—Photograph of skin surface biopsy tested with non-specific esterase reaction. Two areas of increased density can be seen, corresponding to PL papules and due to increased hydrolytic activity in these sites and the deposition of indigo reaction product (× 3).

DISCUSSION

The cells within the epidermis that failed to incorporate tritiated proline corresponded with the eosinophilic cells seen within the Malpighian layer in 72% of our biopsy material, and were interpreted as dead or dying cells. Furthermore, we also interpret the finding of scattered individual Malpighian cells showing reactivity for the hydrolytic enzymes as indicating the presence of incipient cell death within the epidermis. The hydrolytic enyzme activity seen within the parakeratotic scale was very much stronger than that seen in the parakeratotic scale of lesions of psoriasis (Marks, 1972). The findings of focal cell damage or death within the epidermis and intense hydrolytic activity within the parakeratotic scale suggest the possibility that cell damage may play a role in the production of the parakeratotic scale in PL. A similar process may take place in the organ culture of skin when, after 2 or 3 days *in vitro*, the upper part of the epidermis becomes vacuolated, shows increased eosinophilia and transforms into a parakeratotic scale.

In psoriasis the epidermal transit time is decreased 7 or 8 times and an active Malpighian cell therefore travels into the parakeratotic scale after perhaps only 2 days. It is perhaps for this reason that abundant mitochondrial enzyme activity could be identified in the parakeratotic scale from psoriasis, using the skin surface biopsy technique (Marks, 1972). It should be noted that, in this present investigation, a comparable degree of enzyme reactivity was not demonstrable in the scale. Weinstein and Frost (1969) give the autoradiographic labelling index for psoriatic lesions as 22·5%. We were not surprised to find some areas within the inflamed papules of PL that showed raised labelling indices, as this is seen as a non-specific response to epidermal damage in other situations, such as in lichenified eczema (Marks and Wells, 1972). However, the overall labelling index of 15·2% is considerably below that for psoriasis and probably also supports the concept that epidermal damage rather than increased epidermopoiesis is responsible for the parakeratosis. The biopsy taken after injection of a papule some 3 days previously with tritiated thymidine also favours this hypothesis, as the furthest that labelled cells reached at this time was below the level of transformation into

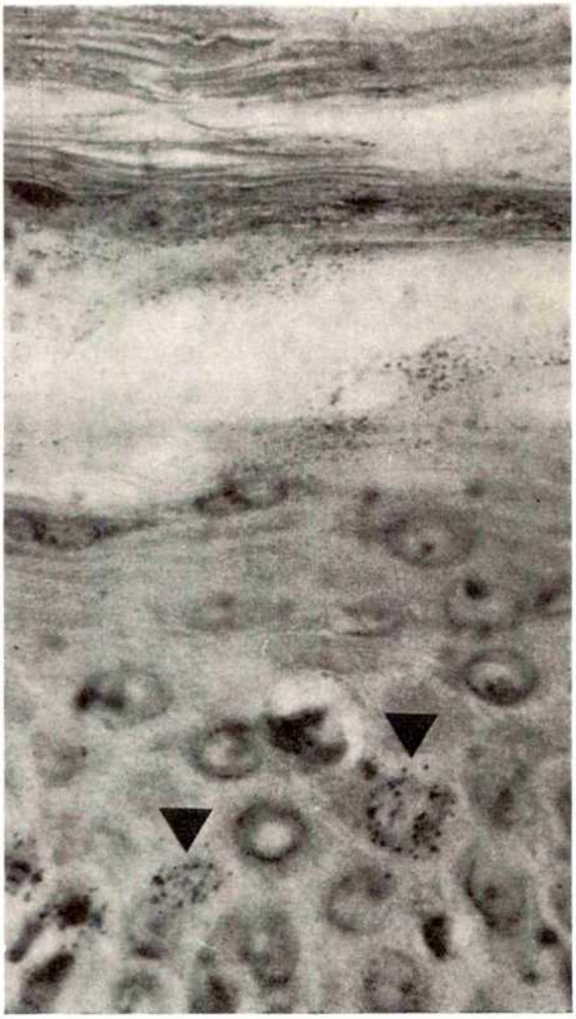

FIG. 4.—Autoradiograph from biopsy of PL papule injected with tritiated thymidine 3 days previously. The triangles indicate the highest points in the epidermis reached by labelled cells (H. and E. × 150).

parakeratotic scale. It is of interest that Christophers and Braun-Falco (1970) questioned whether, after stripping with sticky tape, the parakeratosis that results is due to a high epidermal cell turnover. They believed that cell damage, including altered membrane function of the epidermal cells, might be responsible, and wondered whether this mechanism could be of importance in psoriasis. Our concept, in which we believe that parakeratosis in PL results from cell damage rather than a high rate of epidermopoiesis, is similar to that proposed by Christophers and Braun-Falco (1970), and perhaps the pathogenesis of the parakeratosis of PL differs from that of psoriasis not in kind but in degree.

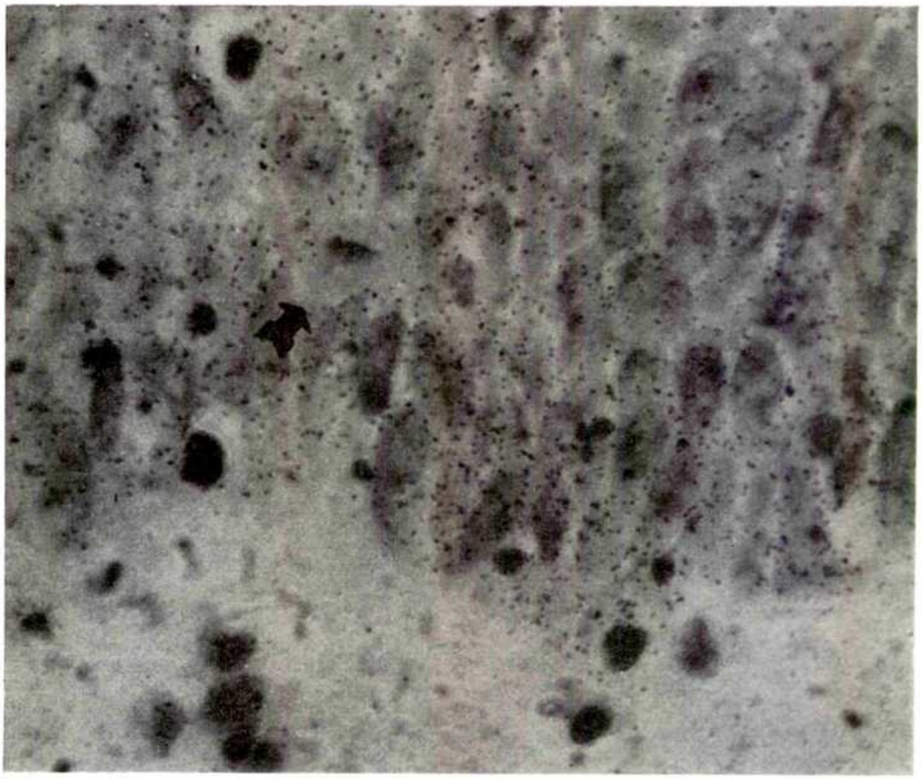

FIG. 5.—Autoradiograph from biopsy of PL papule incubated in the presence of tritiated proline for 4 hours. The arrow indicates an epidermal cell that has not incorporated the proline (H and E. × 150).

We believe that the changes seen within the epidermis in PL, although of interest as a model for one type of parakeratosis, are secondary to some event that seems to involve both the epidermis and the upper dermis simultaneously. It is unknown how the epidermal damage is caused in PL, but we believe that the invasion of the epidermis by inflammatory cells may be important in this respect.

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TOXOPLASMOSIS AND PITYRIASIS LICHENOIDES

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Summary.—Eleven patients with pityriasis lichenoides have been tested for toxoplasmosis. In 6 of them the complement fixation test, the intradermal test with toxoplasmin and the Sabin–Feldman test were repeatedly strongly positive. The same patients reported continuous occupational animal contact. Both the toxoplasma-positive patients and 3 negative patients were submitted to treatment with pyrimethamine. All 6 toxoplasma-positive patients responded very favourably: 3 were completely cured, 75% clearance of lesions was obtained in 2, and 50% in one. The 3 toxoplasma-negative patients failed to improve.

The authors believe that *Toxoplasma gondii* infection may play an aetiological role in pityriasis lichenoides. More observations are needed in order to corroborate or to reject this supposition.

Skin manifestations in toxoplasmosis are non-specific and of varied clinical appearance. A wide variety of skin changes, including macular, maculo-papular, nodular and haemorrhagic eruptions, have been reported. Their pathogenic mechanism is not clear, but there is strong evidence that they may be of toxi-allergic origin. *Toxoplasma gondii*, the causative agent of toxoplasmosis, has never been found in the skin lesions.

A skin eruption of pityriasis lichenoides varioliformis type has been reported by Andreev *et al.* (1969). Pityriasis lichenoides (PL) is a rare and rather poorly defined skin disease. Histologically it is similar to allergic vasculitis. Consequently there has often been a temptation to regard PL as a reaction of hypersensitivity to an infective organism.

Sporadic small epidemics of PL have been reported (Marshall, 1956; Korovitski *et al.*, 1964), and their occurrence supports the presumption of the possible role of an infective agent, at least in some patients with PL. It certainly may be significant that all reported epidemics have occurred in cattle breeding countries. However, all attempts to isolate an infective agent have so far failed.

We believe that the present study is justified by the following observations: (1) Many patients with PL reported continuous animal contact; (2) sporadic outbreaks of PL occurred in cattle breeding regions; (3) our own observation of a patient with toxoplasmosis and a skin eruption of the PL type.

PATIENTS AND METHODS

Three different groups of persons are included in this study:

Group I: Patients with PL—11.
Group II: Patients with psoriasis vulgaris—30.
Group III: Presumably healthy individuals—210.

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Groups II and III served as controls. All patients and healthy persons involved in this study have been submitted to the following tests: (1) Complement fixation test; (2) intradermal test with toxoplasmin.

In addition, the Sabin–Feldman test has been performed in all patients with PL. The patients and persons in Groups II and III have been tested only once. The patients with PL have been tested several times. The tuberculin test was performed on 7 patients with PL.

RESULTS AND DISCUSSION

In 6 of the 11 patients (54·5%) with PL the complement fixation test, the intradermal test with toxoplasmin and the Sabin–Feldman test were strongly positive. Four patients with psoriasis vulgaris (of 30 tested) showed a slightly positive complement fixation test (14·3%). In the group of healthy individuals, 16·7% gave slightly or moderately positive reactions either to the complement fixation test or to the intradermal test.

The number of patients with PL is too small to allow an exact statistical evaluation of the figures. The fact remains, however, that more than half the patients with PL who passed through the Department of Dermatology in the course of the last 2 years (when this investigation began) were repeatedly toxoplasma-positive.

Two important relationships were established in the patients with PL: (1) All toxoplasma-positive patients gave a long history of the disease (5 to 30 years). The initial eruption in the toxoplasma-negative patients had developed only a few months earlier. (2) All toxoplasma-positive patients were in continuous contact with animals; 3 of them were butchers, one was a shepherd, one was an attendant at a zoo, and one reported the habit, whilst cooking, of tasting the raw minced meat. Animal contact in the remaining 5 patients was inconstant and inconclusive.

The tuberculin test in the 7 patients tested was negative.

The skin eruption consisted of disseminated erythematous, papular and squamous lesions, 3–5 mm in diameter. In 2 patients (toxoplasma-positive) there were indurated papules of a dark violaceous colour, haemorrhagic and papulo-necrotic lesions, very similar to the vascular allergides or to PL varioliformis of Mucha.

Three toxoplasma-positive patients had systemic symptoms, consisting of joint pains, constant headache, loss of appetite, tachycardia and chest pains.

The 6 patients with positive serological and skin reactions, as well as 3 patients with negative serology, were submitted to treatment with pyrimethamine (Daraprim) according to the following scheme: 2 tablets per day for 5 days, repeated after an interval of 20 days. After 6 months a second course according the same pattern was given.

Complete cure was achieved in 3 patients. Partial remission, with subjective and objective improvement, including clearance of lesions (75% of the lesions cleared completely) occurred in 2 patients. Moderate improvement, with 50% clearance of lesions, was observed in one patient. The 3 patients with negative toxoplasma tests failed to improve.

The aetiology of PL is still obscure. Repeated attempts to isolate a specific organism have failed. It seems, however, that, at least in some cases, the aetiological role of a bacterial or viral organism cannot be excluded. What are the chances of toxoplasmosis being an aetiological factor in PL?

In 6 of 11 patients continuous animal contact was established. This is epidemiological evidence of considerable importance, since such a relationship does not exist in other groups of skin disorders with similar morphology or pathology. The same 6 patients were strongly positive to toxoplasma tests and all of them responded favourably to the treatment with pyrimethamine. On the other hand, patients with PL who were serologically toxoplasma negative were completely resistant to this treatment.

The comparison with the 2 control groups is also significant. Psoriasis is another erythemato-squamous dermatosis of unknown aetiology, with the same age–sex distribution as PL. Animal contact has been established in 10–15%, which exactly corresponds to the percentage of toxoplasma-positive patients. The same is valid for the persons in Group III. Again, the percentage of animal contact or rural residence corresponds to the percentage of toxoplasma-positive individuals. Epidemiologically there is a basic difference between the patients from Group I and those from Groups II and III. There is a direct relationship between animal contact—pityriasis lichenoides—toxoplasmosis, which can be expressed in the following figures: 54·5% of the patients with PL report occupational animal contact; 100% of those are strongly positive to all tests for toxoplasmosis. For the population at risk the animal contact is about 15%, and of those 15% only 34·0% show positive toxoplasma tests of variable intensity (strong, moderate and slight).

The number of patients with PL is too small for these figures to have absolute statistical value. For such a small number, a statistical error of ±30% is admissible, but, even with this correction, the apparent relationship between PL and toxoplasmosis remains quite impressive.

With this communication we should like to encourage similar investigations in hospitals and clinics with a bigger number of patients with PL at their disposal. Only in this way will more light be thrown on the problem of the relationship between PL and toxoplasmosis.

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THE ULTRASTRUCTURE OF LICHEN AMYLOIDOSUS WITH SPECIAL REFERENCE TO THE EPIDERMAL CHANGES

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Summary.—The ultrastructure of lichen amyloidosus has been studied with special reference to the epidermal changes. The amyloid deposits often came into close proximity to the lower epidermis and obscured the basal lamina. Occasionally, breaks in the basal lamina occurred so that amyloid fibrils came into direct contact with the cytoplasm of basal cells. Small deposits of amyloid were sometimes seen in the inter-cellular spaces between basal cells. Focal areas of liquefaction degeneration were observed within a number of basal cells. A few keratinocytes in the upper epidermis contained deposits of amyloid-like fibrils. It is suggested that these amyloid-like fibrils probably represent degenerate tonofilaments rather than true amyloid.

In 1965 Hashimoto *et al.* suggested that the amyloid fibrils in lichen amyloidosus were secreted by fibroblasts and then deposited in the papillary dermis. Since then several other investigators (Ebner, 1968; Rodermund and Klingmüller, 1970; Pierard and Kint, 1971; Hashimoto and Onn, 1971) have stressed the role of dermal fibroblasts in the pathogenesis of lichen amyloidosus. In a light-microscopic study of macular amyloidosis (Black and Wilson Jones, 1971) it was suggested that damage to the lower epidermis may be the primary pathology in macular amyloidosis, and that degenerating epidermal cells could be converted into amyloid deposits. A recent enzyme histochemical study of lichen amyloidosus (Black, 1971) has indicated further that the epidermis is likely to be involved in the histopathogenesis of lichen amyloidosus. The purpose of the present communication is to present the ultrastructural findings in lichen amyloidosus, with special reference to the changes in the epidermis.

MATERIALS AND METHODS

Twelve patients with histologically proven lichen amyloidosus were studied. Four had the classical papular form of the disease on the lower legs, and 8 had the macular type on the upper back. Further biopsies were taken from an affected site, and each specimen was sliced into small blocks (about 1–2 mm^3). The tissue was fixed for up to 2 hours in cold 2% glutaraldehyde buffered with 0·1 mol/l sodium cacodylate, followed by 45 min in cold 1% osmium tetroxide in Palade buffer. The tissue was then dehydrated through an ethanol series, embedded in Epon 812 and sectioned on an LKB Ultratome 1 with glass knives. Sections were collected on 400 mesh copper grids, stained with 1·5% aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined on an AEI EM6B electron microscope, using an accelerating voltage of 60 kV.

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RESULTS

The ultrastructural findings were essentially the same in both macular and papular lichen amyloidosus, although in the papular type the amyloid deposits tended to be larger and more extensively distributed in the papillary dermis. The upper part of the amyloid deposits was often situated in close proximity to the lower epidermis. In some areas the basal lamina could not be clearly identified (Fig. 1), or ceased abruptly, allowing the amyloid fibrils to come into direct contact with the cytoplasm of basal epidermal cells. Cytoplasmic processes of fibroblasts were present within the larger amyloid deposits, but could not be identified amongst amyloid fibrils close to the cytoplasm of basal epidermal cells. In several sections focal areas of vacuolar degeneration could be identified in the lower part of the cytoplasm of a number of basal epidermal cells (Fig. 2). This vacuolar degeneration varied in severity from a slight reduction in the numbers of tonofilaments present to a total loss of the tonofilaments and other cytoplasmic structures. In other areas amyloid fibrils could be identified in the inter-cellular

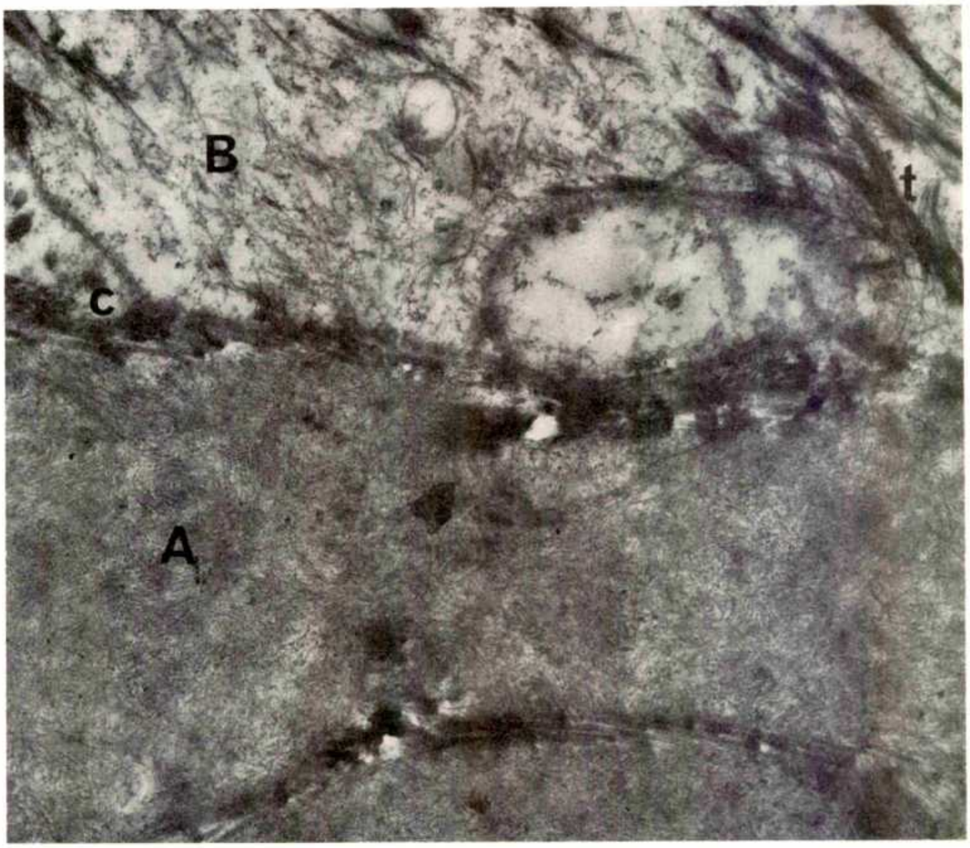

FIG. 1.—Amyloid fibrils (A) come into direct contact with the cytoplasm of a basal epidermal cell (B). The basal lamina cannot be clearly identified and is obscured by collagen fibres (C) and amyloid fibrils. *t* = tonofilaments (× 60,000).

spaces between basal epidermal cells (Fig. 3). Deposits of amyloid-like fibrils were sometimes found within individual keratinocytes in the upper epidermis (Fig. 4). These filaments were wavy, curled, and often densely packed.

DISCUSSION

This ultrastructural study of lichen amyloidosus has provided further confirmatory evidence that the epidermis is damaged at some stage in the disease process. We have confirmed the observations of Ebner (1968) and Hashimoto and Onn (1971) that in several areas the deposits of amyloid came into close opposition with basal epidermal cells. Occasionally, breaks in the basal lamina were observed, and this allowed fibrils to come into direct contact with the cytoplasm of basal cells.

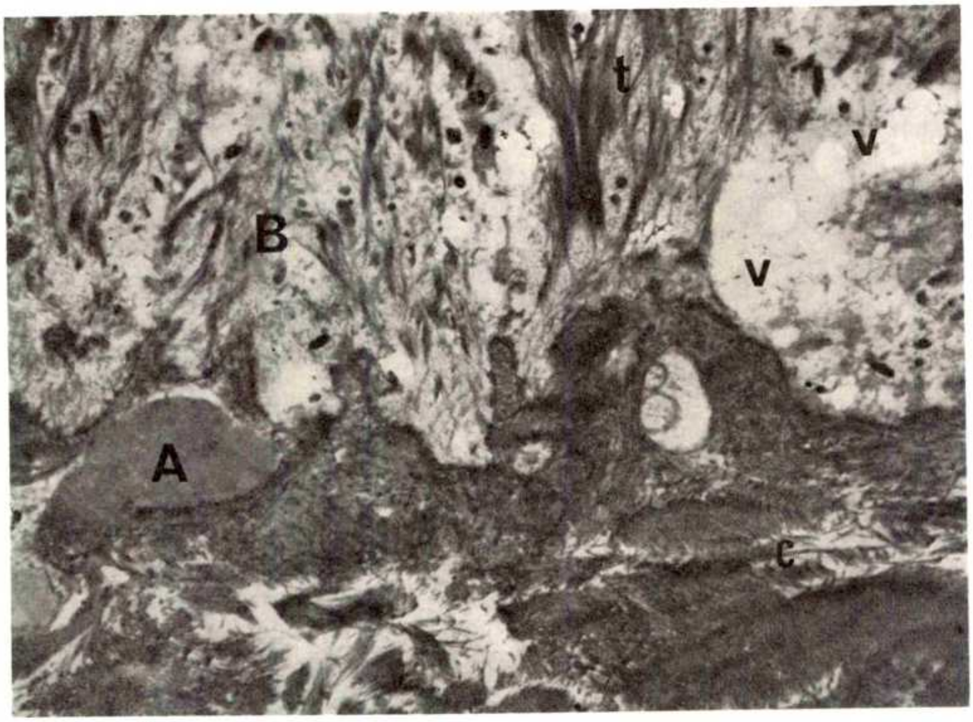

FIG. 2.—A small deposit of amyloid (A) is adjacent to the cytoplasm of a basal cell (B). The cytoplasm of another basal cell is showing severe vacuolar degeneration (V), with complete loss of tonofilaments and cytoplasmic structure. C = collagen fibres; *t* = tonofilaments (× 6000).

Like Ebner (1968), Hashimoto and Onn (1971) and Revuz *et al.* (1972), we noted the presence of amyloid-like fibrils within some upper epidermal keratinocytes. Ebner, and Revuz *et al.* concluded that these intra-epidermal deposits consisted of amyloid fibrils. Hashimoto and Onn described 2 types of filamentous structures within keratinocytes. The first was straight and about 10 times as thick as an average amyloid fibril, with a dense central core. The second type was wavy, curled and densely packed, with a diameter slightly thicker than tonofilaments.

We were unable to identify any of the thicker straight fibrils and observed only wavy, curled and densely packed fibrils, which were similar in size to tonofilaments. True amyloid fibrils in the papillary dermis lacked this wavy pattern and were less densely packed. It is suggested, therefore, that these wavy fibrils represent abnormal or degenerative tonofilaments. However, the possibility of true

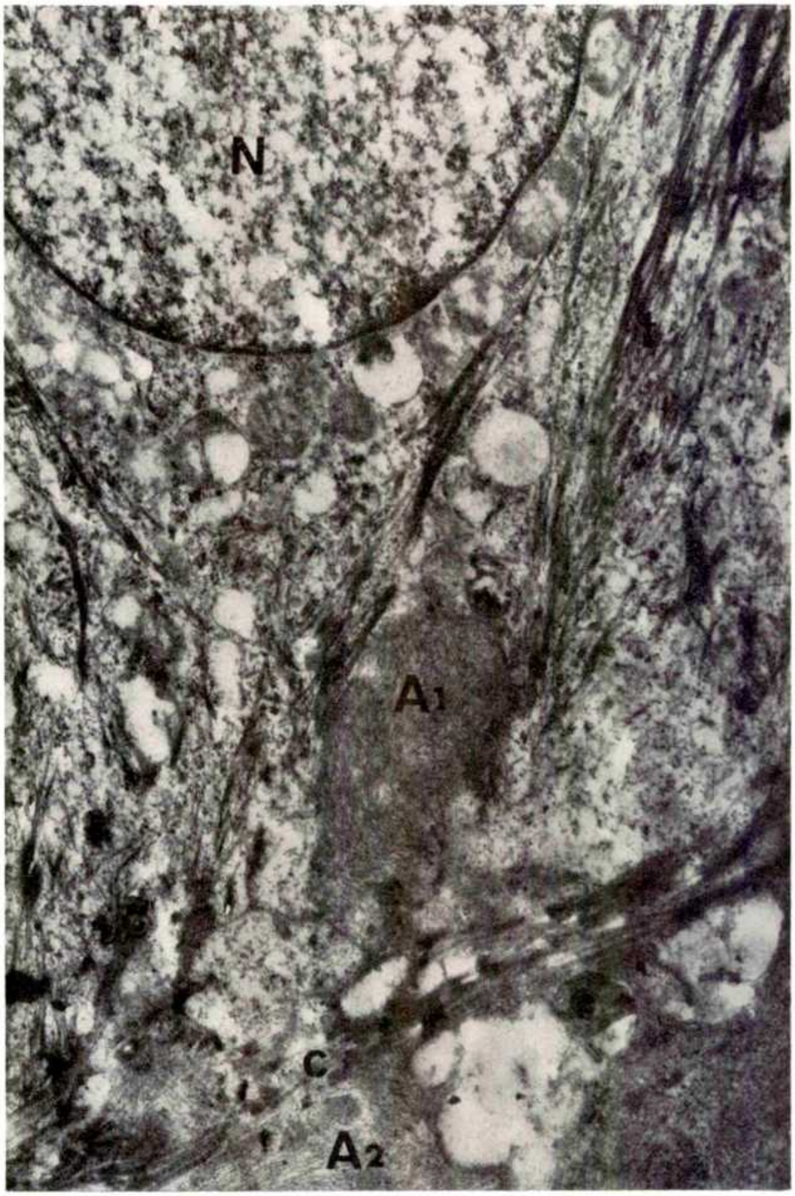

FIG. 3.—A small deposit of amyloid (A1) lies in the inter-cellular space between 2 basal cells. The basal lamina is obscured by collagen fibres (C) and amyloid fibrils (A2). N = nucleus of basal cell; *t* = tonofilaments; C = collagen (× 30,000).

amyloid fibrils also being present within these intra-epidermal deposits cannot be excluded entirely.

In a light-microscopic study Freudenthal (1930) suggested that in lichen amyloidosus minute deposits of amyloid were being extruded upwards through the epidermis. Like Hashimoto and Onn (1971) we also observed small deposits of amyloid situated in the inter-cellular spaces between basal cells. It is possible, therefore, that this inter-cellular amyloid could eventually be eliminated through the epidermis.

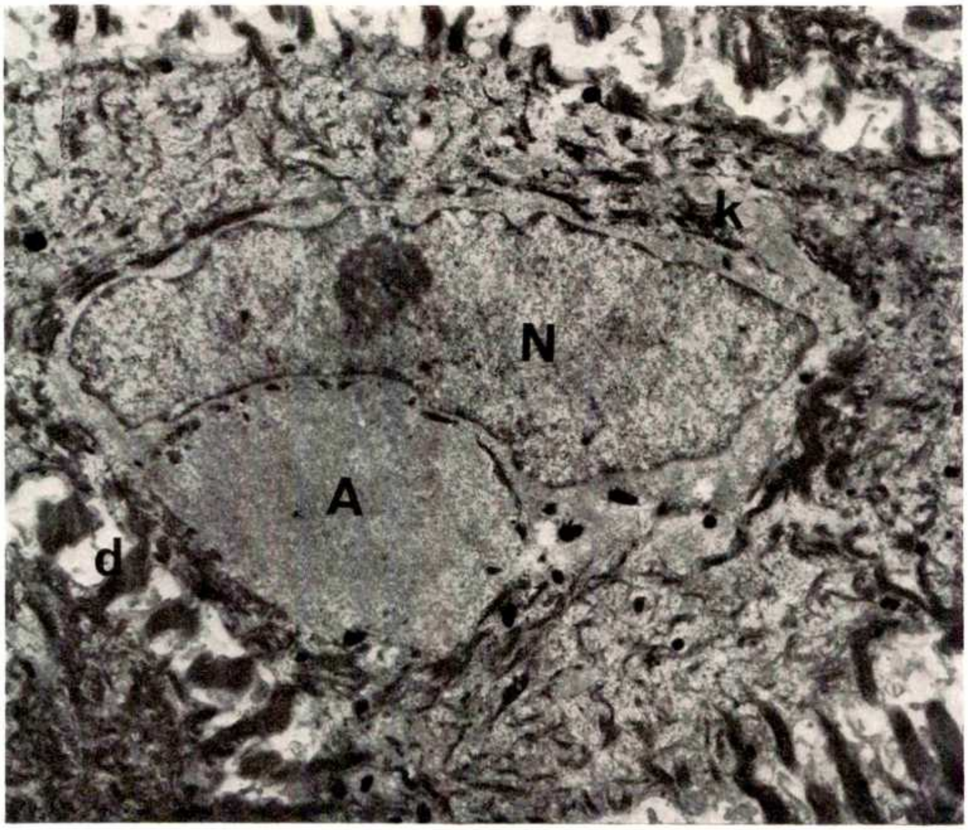

FIG. 4.—A mass of amyloid-like fibrils (A) is situated in a keratinocyte at the level of the stratum spinosum. N = nucleus of keratinocyte; d = desmosomes; K = keratohyaline granules (× 22,000).

Well-marked changes of liquefaction degeneration within basal epidermal cells could be detected in some areas. It is possible that amyloid deposits situated below the epidermis could initiate this change within the basal cells by means of a nutritional or direct pressure effect. Although cytoplasmic processes of fibroblasts could not be clearly identified close to the lower epidermis, fibroblasts were found within the deeper amyloid deposits. Recently Glenner *et al.* (1971a, b) have shown that some types of amyloid can be synthesized from light polypeptide chains. It is not inconceivable that basal epidermal cells could partake directly in the formation of amyloid, perhaps by synthesizing light polypeptide chains into amyloid. It is hoped to be able to investigate this possibility further in the future by means of immunofluorescent techniques.

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FAMILIAL LICHEN AMYLOIDOSIS

REPORT OF 19 CASES IN 4 GENERATIONS OF A CHINESE FAMILY IN MALAYSIA

K. RAJAGOPALAN AND C. H. TAY

Summary.—Nineteen members in 4 successive generations of one Chinese family in Malaysia were found to have classical lichen amyloidosis without systemic amyloid involvement. The disease was transmitted as an autosomal dominant trait with variable penetrance. Sex ratio was equal and the onset was around the age of puberty. The extent and severity of the lesions tended to increase with age. No patient subsequently developed the systemic form of amyloidosis.

The present findings further strengthen the genetic theory of lichen amyloidosis.

In recent years, primary cutaneous amyloidosis or lichen amyloidosis (LA) has been the subject of numerous reports (Porto, 1959; Ruszcak and Wozniak, 1961; Malak and Smith, 1962; Porto *et al.*, 1963; Cortes, 1969; Dugois *et al.*, 1965; Hashimoto *et al.*, 1965; Chow and Burn, 1967; Champion, 1967; Saltzer *et al.*, 1968; Wolf and Tolmach, 1969; Tay and Da Costa, 1970; Black and Wilson Jones, 1971).

Many hypotheses have been postulated regarding the aetiology of lichen amyloidosis, but none has been convincingly proven (Ruckavina *et al.*, 1956; Porto *et al.*, 1963; Wolf and Tolmach, 1969; Black and Wilson Jones, 1971). Amongst these hypotheses is the genetic theory which is supported by a few familial examples (Isaak, 1950; Sagher and Shanon, 1963; De Souza, 1963; Tay, 1971). These reports, however, concerned small families (4 or fewer members), in which the mode of inheritance of the disease could not be reliably established.

In this paper we wish to report one Chinese family in Malaysia, with 19 affected members in 4 successive generations with the classical skin condition. The clinical features, modes of disease transmission and investigations are presented and discussed.

CLINICAL MATERIAL

The Proposita or Case 1 (Fig. 1, arrowed, IV, 2)

This Chinese woman aged 21 was referred to the Skin Clinic, General Hospital, Johore Bahru, Malaysia, in March 1970 for a skin condition of about 4 years' duration. Born and bred in the southern state of Johore, Malaysia, she had been in excellent health. She seldom travelled out of the country.

The lesions appeared gradually and symmetrically on the normal skin surface in front

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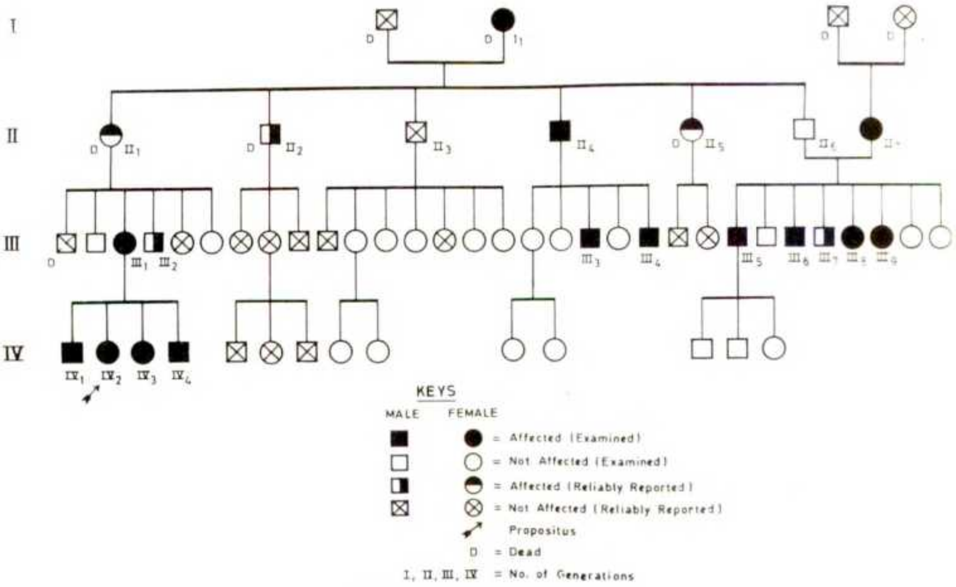
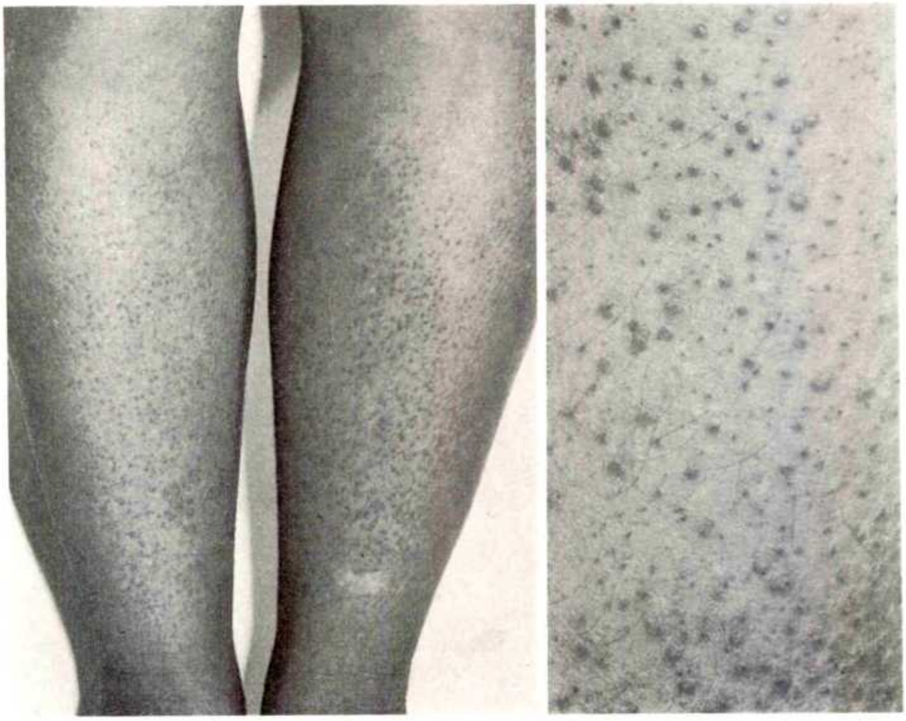


FIG. 1.—The family tree.



(a) (b)

FIG. 2.—(a) Papular lesions of lichen amyloidosis on both pretibal regions of the lower legs. (b) Closer view of the papular lesions.

of both shins and were accompanied by intense pruritus, but with no systemic symptoms. The earlier cutaneous lesions were mostly discrete, small, yellowish papules; but, later, these clustered closely together to form confluent plaques. Papules spread from the lower legs to the thighs and then to the extensor aspects of the forearms.

There was no past history of contact with chemicals, exposure to strong light, medications or external injuries, and she had had no significant past systemic illnesses.

On physical examination, she was essentially normal. There were no signs of macroglossia, visceromegaly, lymphadenopathy, nephrotic syndrome, cardiac failure or other evidence of systemic amyloidosis.

Local examination revealed a large number of brownish-yellow hyperkeratotic papules, 1–3 mm in diameter, hemispherical in shape, slightly elevated and distributed over the pretibial regions of both legs (Fig. 2A, B). Most papules were discrete, but, in some areas, they formed confluent plaques. Similar papular lesions were found on the thighs and the forearms. None was present on the face, trunk or mucous membranes. Scratch marks with lichenification were seen around these lesions, but there were no purpura, ulceration, nodule formation, hair changes or sensory loss.

Most haematological and biochemical results were within normal limits and these were as follows: complete RBC and WBC counts, haematocrit, peripheral blood film, platelet count, reticulocyte count, bone marrow, blood sedimentation rate, serum electrolytes, blood urea, liver function tests, routine urinalysis, Bence Jones proteins, Congo-red test, rectal biopsy, and radiological examinations of the chest and abdomen. Serum albumin was 4·0 g%, serum globulin was 3·4 g%, of which α_1 was 0·2 g%, α_2 0·89 g%, β 0·9 g% and γ 1·5 g%. Serum immunoglobulin estimation showed IgG 1200 mg%, IgA 176 mg% and IgM 98 mg% (all normal values for the same age and sex in Singapore).

Skin biopsy.—Light microscopic examination of the skin lesion taken from the pretibial region revealed marked hyperkeratosis of the epidermis with some papillomatosis and downward proliferation of the rete pegs. The upper dermis showed homogenization of the collagen, with widening of the dermal papillae in some parts. The deposits in the papillary dermis stained metachromatic purplish colour with methyl violet and stained strongly with Congo-red, crystal violet, toluidine blue and fluoresced with thioflavin-T. The appearance was therefore consistent with that of lichen amyloidosis.

Family Study (see Table I)

From the proposita (Case 1), we were able to trace 18 other members in 4 successive generations of her family with lichen amyloidosis. The family tree is shown in Fig. 1 and the detailed findings of all the 19 affected individuals are summarized in Table I.

Comment on the family study.—The numbers of individuals affected in the 4 successive generations were 1, 5, 9 and 4 respectively. Ten were males and 9 were females. The ages of affected members ranged from 18 to 86 years, the majority being in the third decade of life. The age of disease onset was between 15 and 20 years and most of the patients had had the lesions for well over 30 years, the longest being 66 years. Of the 19 cases, 14 were examined personally and had histological evidence of lichen amyloidosis; the rest were reliably reported by close relatives to have the disease. None had symptoms, signs or other evidence of systemic amyloidosis. Pruritus was present during the initial stage of the disease in all those affected, but the symptom lessened with time. Classical papular lesions were found on the pretibial areas of the legs in almost all the cases and only 2 had isolated macular lesions. Older patients and a few younger members had extensive lesions on both upper and lower limbs as well as the trunk, and the lesions coalesced to form confluent plaques. The lichen amyloidosis persisted for life and showed no remission. None of the 19 cases eventually developed systemic amyloid involvement. In 3 affected members of this family who had died, the cause of death was not related to the amyloid disease.

Case no.	Corres-ponding no. as shown Fig. 1 (Generation, number)	Relation to proposita	Age and sex	Age of onset	Disease duration (years)	Sites of lesions	Types of lesions	Examined (E) or reliably reported (RR)	Biopsy confirmed	Alive/dead
1	IV, 2	Proposita	21F	17	4	Lower legs, forearms	Papules. Few plaques. (Fig. 2(a) and (b)	Examined	+	Alive
2	I, 1	Maternal great-grandmother	86F	20	66	Limbs and trunk	Papules and plaques (Fig. 3)	Examined	+	Dead
3	II, 1	Maternal grandmother	Died 42F	18+	24	Lower legs	Papules	Reliably reported	−	Dead
4	III, 1	Mother	41F	16	25	Lower legs, thighs, and arms	Papules	Examined	+	Alive
5	III, 2	Maternal uncle	42M	15+	27	Lower legs	? Papules	Reliably reported	−	Alive
6	IV, 1	First brother	26M	20	6	Forearms, lower legs	Papules	Examined	+	Alive
7	IV, 3	Sister	18F	17	1	Lower legs	Papules	Examined	+	Alive
8	IV, 4	Second brother	19M	17	2	Lower legs	Papules	Examined	+	Alive
9	II, 2	First maternal great-uncle	Died 67M	20+	45+	Arms and legs	Papules	Reliably reported	−	Dead
10	II, 4	Second maternal great-uncle	59M	20+	39	Lower legs	Papules	Examined	+	Alive
11	III, 3	Second maternal great-uncle's first son	27M	17	10	Lower legs	Papules and macules	Examined	+	Alive
12	III, 4	Second maternal great-uncle's second son	22M	18	4	Both shins	Macular lesion	Examined	+	Alive
13	II, 5	Maternal great-aunt	Died 48F	20	28	Lower legs	Papules	Reliably reported	−	Dead
14	II, 7	Wife of third maternal great-uncle (II, 6)	60F	15+	45	Arms, legs and trunk	Papules and plaques	Examined	+	Alive
15	III, 5	First son of third maternal great-uncle and aunt (II, 7)	36M	19	17	Arms, legs and trunk	Papules and plaques	Examined	+	Alive
16	III, 6	Second son of II, 7	29M	20	9	Arms and lower legs	Papules	Examined	+	Alive
17	III, 7	Third son of II, 7	21M	19	2	Lower legs	Macular lesion	Reliably reported	−	Alive
18	III, 8	First daughter of II, 7	31F	20	11	Lower legs and thighs	Papules and macules	Examined	+	Alive
19	III, 9	Second daughter of II, 7	26F	18	8	Lower legs	Papules and some macules	Examined	+	Alive

Mode of Hereditary Transmission

From the proposita or Case 1 (Table I) (Fig. 1, IV, 2), we were able to trace the rest of the family tree. Thus, as shown in Fig. 1, the proposita's great-grandmother, Case 2 (Table I, or Fig. 1, I, 1) was the origin of the disease in this family. She had unaffected parents, and her husband had no skin disorders. She came from southern China during her teens and developed extensive LA (Fig. 3) at the age of 20, 66 years ago. Four of her 6 siblings were similarly affected. They were:

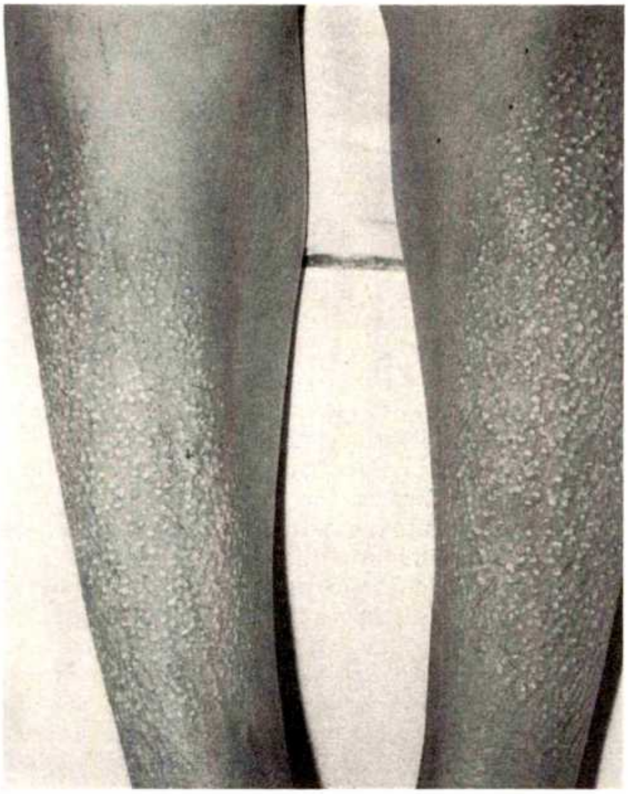


FIG. 3.—Plaques of lichen amyloidosis in Case 2.

Case 3 (Table I) (Fig. 1, II, 1), who had one affected daughter (Case 4) and one affected son (Case 5). The former (Fig. 1, III, 1) had all her 4 siblings affected (Cases 6, 7, 8 and 1) (Fig. 1, IV, 1 to 4). Hence the transmission is that of autosomal dominant trait with complete penetrance.

Case 9 (Table I) (Fig. 1, II, 2), the first son of Case 2, was reliably reported to have LA, but all his children and grandchildren were unaffected.

Case 10 (Table I) (Fig. 1, II, 4) had 2 affected sons (Cases 11 and 12), but the granddaughters were not affected.

Case 13 (Table I) (Fig. 1, II, 5), the second daughter of Case 2, was reported to have LA, but not her 2 siblings.

The above 3 families, thus showed incomplete penetrance of the disease.

Case 14 (Table I) (Fig. 1, II, 7) was the affected wife of a non-involved son of Case 2 (Figure 1, II, 6). She appeared to be a sporadic case with unaffected parents or relatives.

However, 5 of 8 siblings (Cases 15 to 19) (Fig. 1, III, 5 to 9) had extensive and severe lichen amyloidosis. Her younger grandchildren were not affected. An autosomal dominant inheritance is thus demonstrated, with Case 14 as the most likely second source of this family. The other unaffected son of Case 2 (Fig. 1, II, 3) had no affected cases in his family.

DISCUSSION

To date, only 4 familial examples of lichen amyloidosis (LA) have been documented in the literature. Isaak (1959) first reported 2 siblings in America with LA and psoriasis. Sagher and Shanon (1963) found 3 members of a Russian family in 3 generations with this disease. In South America, De Souza (1963) reported the third family with 4 affected siblings and, recently, Tay (1971) from Singapore found a mother and her daughter with primary amyloidosis of the skin. As the cases reported in these families are small, the mode of disease transmission is far from clear.

Our family, therefore, is the largest so far recorded. It shows conclusively that lichen amyloidosis can be transmitted as a Mendelian autosomal dominant trait, with variable penetrance. In some instances the penetrance may be complete (e.g. siblings of Cases 4 and 14), in others, incomplete.

Another interesting genetic aspect of this family is that the skin disease came from 2 different sources quite by chance—Case 2 and Case 14. The high penetrance in the family members of Case 14, who had extensive and severe skin lesions of LA (Cases 15 to 19), may well suggest that her unaffected husband, the son of Case 2 (Fig. 1, II, 6), was probably an unsuspected heterozygote.

The finding of this family favours the genetic theory of LA, since we have excluded known causes like trauma, exposure to chemicals, strong light, medications and other associated systemic conditions. The fact that none in this family eventually developed primary systemic amyloidosis even although they had the disease for as long as 60 years, disproves the suggestion that LA is a *forme fruste* of primary systemic amyloidosis (Curtis, 1967). There is no evidence of an inborn error of metabolism in this and other studies (Tay and DaCosta, 1970); nor is there any convincing evidence of a disorder of immunological mechanisms. The former condition was suggested earlier by Wolf and Tolmach (1969) and the latter was mooted because of the abnormal proliferation of the lymphoreticular or plasma cell series commonly present in systemic amyloidosis (Cohen and Calkins, 1959; Gafni *et al.*, 1966). Abnormal serum α-2-globulins reported in families by Rukavina *et al.*. (1956) and Porto *et al.* (1963) were not detected. Serum immunoglobulins, estimated in some of our cases, were within normal limits.

The racial susceptibility to this disease thus reflects the role of a genetic factor. It is rare amongst the Europeans and North Americans (Hadida and Streit, 1961; Champion, 1967; Brownstein and Helwig, 1970), but is commoner in South Americans (Porto, 1962; Di Prisco *et al.*, 1963; Wolf and Tolmach, 1969; Cortés, 1969), Israelis (Shanon and Sagher, 1970) and Asians such as the Chinese in Singapore and Malaysia (e.g. our family) (Tay and DaCosta; 1970; Kutty *et al.*, 1970), the Malays (Tay and DaCosta, 1970), the Indians (Banerjee and Dutta, 1970; Black and Wilson Jones, 1971), the Indonesians (Harahap and Hutapea, 1970) and the Japanese (Anekoji and Irisawa, 1961).

Although primary cutaneous amyloidosis is frequently encountered in Asians residing in Singapore (as shown by Leong *et al.*, 1971, who collected a record of

142 cases within a 3-year period), the systemic form of amyloidosis is rarely seen. Only a single case of primary systemic amyloidosis has been recorded locally (Tay, 1970). Because of its rarity, this patient presented a diagnostic problem.

The reasons for the sharp contrast of amyloid disorder pattern in this region with those seen in the West are not well known and remain to be determined.

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A QUANTITATIVE STUDY OF ERYTHRASMA LESIONS

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Summary.—Factors involved in the formation of erythrasma lesions are discussed. In the axillae and groins, the presence of scaling was a significant forerunner of the appearance of a fluorescent lesion. The total bacterial count, the counts of both fluorescent and non-fluorescent diphtheroids, were highest in areas showing both fluorescence and scaling, but the number of micrococci did not differ significantly between affected and non-affected sites.

THE incidence of erythrasma in a hospital for the mentally subnormal has been studied extensively over a period of 4 years. Erythrasma is a superficial infection of the skin, resembling tinea, with lesions which characteristically fluoresce coral red when viewed by Wood's u.v. light. In temperate climates, lesions occur predominantly in the intertriginous sites such as the axillae, groins and toewebs, and mild subclinical cases are frequently seen, diagnosed by their typical fluorescence. In tropical and subtropical areas, more widespread lesions may be seen, and any part of the skin may be involved.

The majority of the male patients in the psychiatric hospital have been examined on several occasions, some up to 10 times, during the 4-year study period, and it has been possible to follow to some extent the course of evolution of erythrasma lesions. Records were available for a total of 3183 samples from these patients, and examination of the samples showed that scaling was apparently an important factor prior to the appearance of a fluorescent lesion (see below). However, it was thought possible that the number of fluorescent organisms on the site rather than their mere presence was also of primary importance in the aetiology of erythrasma, and it was decided to carry out a quantitative examination of the bacterial flora of fluorescent and non-fluorescent sites on selected patients in an attempt to define the pathogenic role of these fluorescent diphtheroids.

MATERIALS AND METHODS

Male patients at a hospital for the mentally subnormal were examined as described previously (Somerville *et al.*, 1970). After examination for visible evidence of scaling in the axillae, groins and toewebs, by the same clinician as previously, these areas were examined using a Wood's u.v. light for signs of the characteristic coral red fluorescence of erythrasma. (For the purpose of this paper, erythrasma is defined as an area showing both scaling and fluorescence; in the present series there were no fluorescent skin areas which did not also show scaling.) Twenty-six fluorescent sites (5 in the axilla and 21 in the groin) and 51 non-fluorescent sites (11 in the axilla and 40 in the groin) were sampled, using a modification of the quantitative technique described by Williamson (1965): 5 ml of sterile 0·075 mol/l phosphate buffer (pH 7·9) with 0·1% Triton X-100 (Lennig Chemicals Ltd) were placed in a sterile Teflon cylinder applied to the skin site

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TABLE I.—*Effect of Scaling and of the Isolation of a Fluorescent Organism on the Development of Erythrasma at Sites Which Showed No Fluorescence at the Index Examination*

	Incidence of erythrasma at second visit								
	Axillae			Groins			Toewebs		
Condition at index visit	No. examined	% developing erythrasma	Significance*	No. examined	% developing erythrasma	Significance	No. examined	% developing erythrasma	Significance
Scaling present	154	8	$P<0\cdot1\%$	420	20	$P<0\cdot1\%$	1254	17	NS
Scaling absent	1623	1		1114	12		266	13	
Fluorescent culture obtained	462	3	NS	497	12	NS	484	17	NS
Fluorescent culture not obtained	1315	1		1037	15		1036	13	
Scaling present and fluorescent culture obtained	46	4		155	19		421	17	

* NS—no significant difference. $P<0\cdot1\%$ indicates a statistically significant difference.

to be sampled. The area enclosed by the cylinder (4 cm^2) was scrubbed for one minute, using a sterile Teflon rod; the resulting suspension was removed, using a sterile Pasteur pipette and ten-fold dilutions made in phosphate buffer with 0·05% Triton X-110; 5 drops (0·2 ml) of each dilution were inoculated on to 5% horse blood agar and incubated aerobically at 37°C for 48 hours. This method does not permit sampling of the toe spaces.

Organisms in appropriate dilutions were counted and a representative colony of each morphological type was inoculated on to plates of Tissue Culture Medium No. 199 (Wellcome) containing 20% calf serum and 2% agar. These plates were incubated at 37°C for 3 days and examined each day for culture fluorescence. Organisms isolated were identified as *Staphylococcus aureus*, coagulase negative "micrococci" (including members of the genera *Staphylococcus* and *Micrococcus*), *Sarcina* species, diphtheroids Gram-negative bacilli, aerobic sporing bacilli, etc. All organisms producing coral red fluorescence were grouped biochemically, the diphtheroids according to Somerville, (1970) and the micrococci according to Baird-Parker (1963).

RESULTS

Scaling.—Serial analysis of the records was performed so that, when a patient had been seen on 6 occasions, there were 5 intervals in which he might progress from a non-fluorescent to a fluorescent site. Table I shows the subsequent appearance of a fluorescent lesion at a previously non-fluorescent skin area, sites showing fluorescence at the index visit being excluded from the data used for the following visit. The right and left axillae were considered as one site, as were the right and left groins and right and left toewebs. The numbers of sites included in Table I are different for the axillae, groins and toewebs, as the difference in incidence of erythrasma in these 3 areas at the index visit resulted in different numbers being deleted from the analysis.

In the axillae and groins the presence of scaling at the index visit was a significant forerunner of subsequent fluorescence; the mere presence of fluorescent organisms was not (Table I). In the toewebs, where it is possible to divide the amount of scaling present into severe (++) and slight (+), culture of fluorescent organisms was an important factor when slight scaling was present at the index visit (Table II).

TABLE II.—*Percentage Incidence of Erythrasma in Toewebs Which Showed No Fluorescence at the Index Visit*

At index visit	No.	% incidence at second visit	Significance*
Scaling + + culture +	263	18	NS
Scaling + culture +	158	15	
Scaling − culture +	63	13	
Scaling + + culture +	263	18	NS
Scaling + + culture −	398	18	
Scaling + culture +	158	15	$2\% < P < 5\%$
Scaling + culture −	435	8	
Scaling − culture +	63	13	NS
Scaling − culture −	203	12	

*NS = no significant difference: $2\% < P < 5\%$ = difference significant between 2% and 5% levels.

TABLE III.—*Mean Counts of Organisms Isolated from Sites Examined*

	Fluorescent site with scaling	Non-fluorescent site with scaling	Non-fluorescent non-scaling site	Significance*	
				Variance ratio	P
No. examined	26	22	29		
Mean count of all organisms	$7 \cdot 5 \times 10^5$	$2 \cdot 0 \times 10^5$	$1 \cdot 4 \times 10^5$	4·97	1%
Fluorescent diphtheroids	$2 \cdot 4 \times 10^5$	$7 \cdot 3 \times 10^4$	$1 \cdot 2 \times 10^4$	3·54	<5%
Fluorescent Gram-positive cocci	$6 \cdot 2 \times 10^3$	$4 \cdot 9 \times 10^3$	$2 \cdot 2 \times 10^3$	0·98	NS
Non-fluorescent diphtheroids	$4 \cdot 5 \times 10^5$	$1 \cdot 3 \times 10^5$	$7 \cdot 0 \times 10^4$	3·32	<5%
Non-fluorescent Gram-positive cocci	$3 \cdot 8 \times 10^4$	$5 \cdot 6 \times 10^4$	$5 \cdot 7 \times 10^4$	0·31	NS

* NS = not significant. $P < 5\%$; $P = 1\%$ indicate a statistically significant difference.

TABLE IV.—*Significance of Differences in Bacterial Counts between the 3 Groups Studied: Fluorescent Scaling Sites (Group A); Non-fluorescent Scaling Sites (Group B); Non-fluorescent, Non-scaling Sites (Group C)*

Groups compared	A and C		A and B		B and C	
	F†	*P**	*F*	*P*	*F*	*P*
Degrees of freedom	$n_1=1;\ n_2=53$		$n_1=1;\ n_2=46$		$n_1=1;\ n_2=49$	
Total flora	6·39	<2·5%	3·94	NS	0·48	NS
Fluorescent diphtheroids	5·86	<2·5%	1·89	NS	1·21	NS
Micrococci	0·05	NS	0·51	NS	<0·01	NS
Non-fluorescent diphtheroids	4·77	<5%	2·33	NS	0·77	NS

* NS = not significant: $P < 5\%$, P < 2·5% indicates a statistically significant difference.

† F = variance ratio.

Quantitative microbiology.—Three categories of site were examined: the first comprised those showing fluorescence and scaling, defined as erythrasma (26); the non-fluorescent sites were divided into those which showed scaling (22) and those with no obvious scaling (29). Fluorescent diphtheroids were isolated from 100% of fluorescent sites, from 73% of those sites showing scaling but no fluorescence, and from 38% of those sites showing neither scaling nor fluorescence. This isolation rate is higher than that obtained in previous studies, reflecting the improved sampling method: a scrub technique using a non-ionic detergent such as Triton X-100 has been shown to remove 85% of the organisms of the normal flora in one scrub (Williamson, 1965). In 14 sites where no fluorescent diphtheroids were isolated, fluorescent micrococci or *Sarcina* species were found; 4 of these sites showed scaling. In a further 16 sites, both fluorescent micrococci and fluorescent diphtheroids were isolated. However, the fluorescent micrococci showed no increase in incidence or number in either scaling or fluorescent areas.

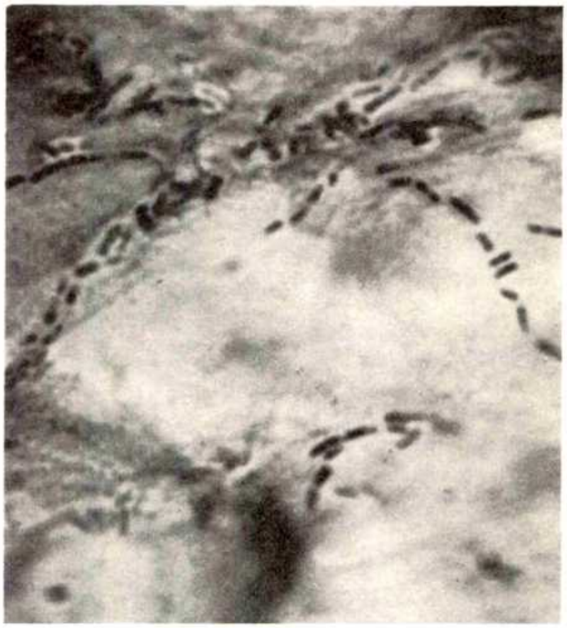

FIG. 1.—Skin surface biopsy of erythrasma lesion showing abundant diphtheroid organisms. Gram stain.

The number of organisms isolated from the different sites varied considerably, but the greatest number was found on the sites with erythrasma (Table III) and the total count, as well as the count of fluorescent diphtheroids and non-fluorescent diphtheroids, was significantly higher on these lesions. However, the magnitude of the differences, especially those in total count, was not great. The counts of Gram-positive cocci did not differ significantly between any of the sites.

Staph. aureus was found in 29% of the areas sampled, but there was no difference either in incidence or quantitative count between the 3 groups studied; in 14 sites the number was more than 10^3 viable units of *Staph. aureus* per cm^{-2} of skin.

Gram-negative bacilli were found in only 5 sites (6%), all of which showed scaling and 4 of which also fluoresced. Gram-positive bacilli other than diphtheroids were not common or numerous, and a *Streptomyces* species was isolated in only one instance, from a non-fluorescent, non-scaling site. Both these groups of organisms have been incriminated in the aetiology of erythrasma (Partridge and Jackson, 1962; Dockx, 1971).

There is, then, a clear difference between 2 of the groups, clinical erythrasma, i.e. sites with both scaling and coral red fluorescence, and the apparently healthy areas with neither scaling nor fluorescence (Table IV). However even in the normal non-fluorescent skin sites, fluorescent diphtheroids do occur and may appear in very large numbers (one healthy site carried 2×10^5 fluorescent diphtheroids per cm^{-2} skin), and this emphasizes the fact that these organisms are members of the normal skin flora. The third group, with scaling but no fluorescence, lies somewhere between the other groups; bacteriologically these areas are rather more akin to the healthy skin areas, but clinically may be diagnosed as erythrasma without the use of u.v. light. In no instance was the quantitative count obtained for these intermediate sites significantly different from those obtained for healthy skin sites. Thus the presence of scaling alone does not appear to affect greatly the number and type of organisms found on the skin. Conversely, however, although fewer organisms were found in the intermediate group than in the group with lesions, there were no statistically significant differences.

The quantitative increase in diphtheroids in lesions could easily be demonstrated by using the skin surface biopsy technique (Marks and Dawber, 1971). Microorganisms are rarely seen in samples from normal skin; but, in lesion samples, abundant diphtheroids were seen (Fig. 1), often exhibiting the pseudomycelial appearance which led previous workers to ascribe erythrasma to fungal infection (for a review see Sarkany *et al.*, 1961).

DISCUSSION

Fluorescent diphtheroids are members of the normal skin flora (Somerville, 1970), and the present quantitative examination confirms this, with the recovery of these organisms from 38% of the healthy sites sampled. In the aetiology of erythrasma other factors are therefore important. Although the fluorescent organisms isolated were grouped biochemically according to Somerville (1970), no single group was involved in the formation of the lesions, and all types could be recovered from the 3 categories of sites.

The examination of records obtained over a period of 4 years from patients in a psychiatric hospital showed that the presence of scaling is important in the subsequent appearance of erythrasma in the axillae and groins. The picture in these sites is most probably one of scaling resulting from chafing and maceration, with subsequent overgrowth of fluorescent diphtheroids producing the typical coral red fluorescent lesion, which may lead to a worsening of scaling. There were, however, several scaling areas from which fluorescent diphtheroids could not be isolated; scaling alone clearly does not lead to a significant increase in the number of microorganisms, and there is obviously some other factor favouring the growth of diphtheroids, specifically fluorescent diphtheroids.

In the toewebs a different mechanism may exist, but it is difficult to define " normal " toes spaces, as 75–90% of the healthy adult population have scaling in the toewebs (Gentles and Holmes, 1957; Somerville, 1966). Fungal infection was not common in this hospital, occurring in less than 10% of the patients.

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EARLIEST FEATURES OF THE STRAWBERRY MARK IN THE NEWBORN

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Summary.—In 2168 infants, 17 cases of strawberry mark were followed from the earliest neonatal stage. Three angiomata began as telangiectatic lesions surrounded by a pale halo; red puncta then appeared on the dilated vessels. They coalesced and became papular, and red plaques were formed. Three angiomata began as a pale patch; telangiectasia then appeared on this patch. One angioma, first seen as a bruise-like macule, transformed into a nodular lesion. One angioma began as a simple and uniform erythema and subsequently became nodular. Two infants already showed early haemangiomata at our first examination. In 5 infants, on whom no abnormality was found within the first week of life, a strawberry mark was found later. Two pale patches in the neonatal stage remained abortive angiomata. The haemangiomatous component developed in the majority of cases between the third and fifth weeks of life.

IN the past 3 years, complete dermatological examinations have been performed weekly on all infants born in the Tokyo Metropolitan Police Hospital, and the early changes of angiomata have been sought. The greater majority of infants were aged between 0 and 6 days at the first examination.

At this neonatal stage, 14 of 2168 infants were suspected of having strawberry marks (Table I, Cases 1–10, 16–19). Eight infants (Cases 1–8) were followed completely, and their records are described. Two infants (Cases 9 and 10) had been placed in the incubator and, when they were first examined at the age of 23 days, papular lesions were already observed. In both cases the lesions subsequently enlarged. Five infants (Cases 11–15), who presented no abnormality during the neonatal stage, and are not therefore included in the 14 cases, came to us later, and the angiomata were found incidentally. In one infant (Case 16) the anaemic patch on the back was accompanied at the age of 44 days by 2 blotches of erythema which disappeared spontaneously at 9 months. This can be considered an abortive angioma; Case 17 was similar. In 2 infants (Cases 18 and 19), which were not followed, the diagnosis remained " suspected strawberry mark ". It is possible, however, that these early changes disappear spontaneously without any visible lesion. If they transform into angiomata they probably become abortive angiomata.

Consequently, 15 infants (Cases 1–15), with a total of 18 strawberry marks, were observed. There were 4 males and 11 females, a ratio of 1 : 2·8. In contrast to generally repeated experience, only 2 angiomata were on the face; 6 angiomata were on the extremities, one on the scalp, one on the genitalia, and 8 on the trunk. Observations were also made on at least 2 angiomata on the back, which remained abortive.

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TABLE I.—*Summary of Observations of the Strawberry Mark*

		First examination			
No.	Sex	Age (days)	Initial lesion	Site	Age at development of angiomatous component
1	M	14	Telangiectasia + pale halo	Trochanteric region	21 days–2·5 months
2	F	11	Telangiectasia + pale halo	Hip	25 days
3	F	5	Telangiectasia + pale halo	Lower leg	17 days
			None	Wrist and toe	Before 1·5 months
4	F	0	Bluish macule	Lip	25 days
5	F	2	Pale patch	Buttock	25 days–1·5 months
6	F	3	Pale patch	Back	23 days–2 months
7	F	1	Pale patch	Back	38 days
8	F	11	Simple erythema	Vulva	1·5–2 months
9	F	23	Telangiectasia + papules	Sternal region	Just before 23 days
10	M	23	Papules	Preauricular region	Before 23 days
11	F	3	None	Abdomen and foot	Before 2 months
12	M	4	None	Forehead	36 days–2 months
13	M	15	None	Finger	33 days
14	F	4	None	Thorax	2 months
15	F	4	None	Back	2 months
16	F	4	Pale patch	Back	Blotchy erythema at 1·5 months
17	F	6	Pale patch	Back	Small papules at 3 months
18	F	2	Telangiectasia	Back	Not followed
19	F	11	Telangiectasia + pale halo	Back	Not followed

Cases 1– 8 Complete record available
Cases 9–10 Elevated strawberry mark from first examination
Cases 11–15 No lesion at first examination; later onset
Cases 16–17 Abortive angioma
Cases 18–19 Suspected strawberry mark

In a large city like Tokyo, it is impossible to expect that all the infants in this series would return to us after discharge if angiomata were noted. However, if the incidence of strawberry mark approximates 0·45%, as Simpson estimated, the number of such infants who dropped out of our survey is probably negligible.

Eight cases with complete records from early neonatal age are presented.

CASE REPORTS

Case 1.—A male infant weighing 4·5 kg, born by Caesarian section of a healthy mother. At the age of 7 days, no abnormality was found on routine dermatological examination. At 14 days, however, a 15 × 20 mm, rose-red, telangiectatic oval patch of erythema surrounded by a pale halo was noticed on the left trochanteric region (Fig. 1). At 21 days, pinhead-sized, flat macules of erythema appeared on the telangiectatic patch, but the pale halo was still visible. By 2·5 months there was a raised, red, 20 × 28 mm plaque (superficial type), and the pale halo was no longer visible (Fig. 2). At 7 months, the angioma was said to be somewhat smaller.

Case 2.—A female infant born normally of a healthy mother. The mother noticed the appearance of an erythema at 9 days. At 11 days, a well-defined triangle of erythema, 26 × 43 mm, was observed on the left hip. The erythema consisted of fine thread-like telangiectasia, more dense on the periphery of the patch, the centre of which remained normal. A discrete pale halo surrounded the erythema (Fig. 3).

At 25 days red papules appeared, mostly on the edge of the patch, and showed partial confluence. The telangiectasia was barely visible, but the pale halo still remained (Fig. 4). A diagnosis of strawberry mark (superficial type) could be readily made at this time. By 45 days the papules in the periphery were confluent to form an annular ring. There were only a few scattered papules in the centre. At 2 months the pale halo was barely visible. At 2·5 months an ulcer appeared in the central portion of the strawberry mark, which had hitherto remained quite normal. The ulcer healed following topical treatment and by 5 months the naevus had almost disappeared.

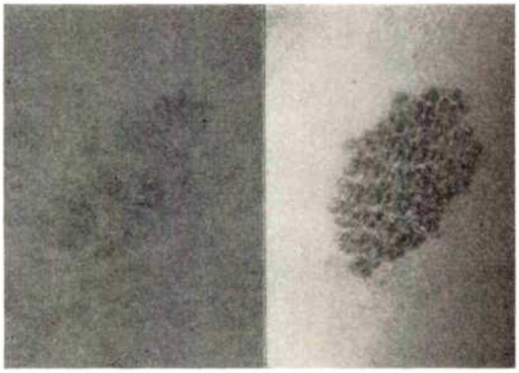
FIG. 1.—Case 1 at 14 days. Telangiectatic erythema on the trochanteric region.
FIG. 2.—Case 1 at 2·5 months.

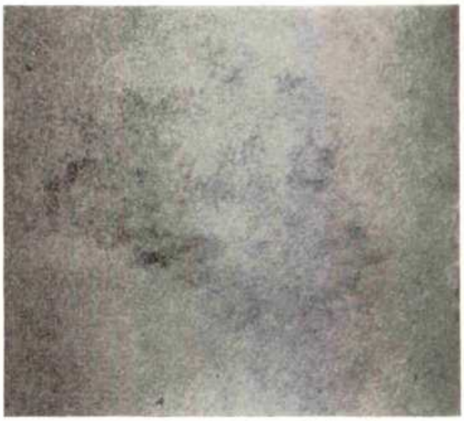
FIG. 3.—Case 2 at 11 days. Telangiectatic area on the hip.

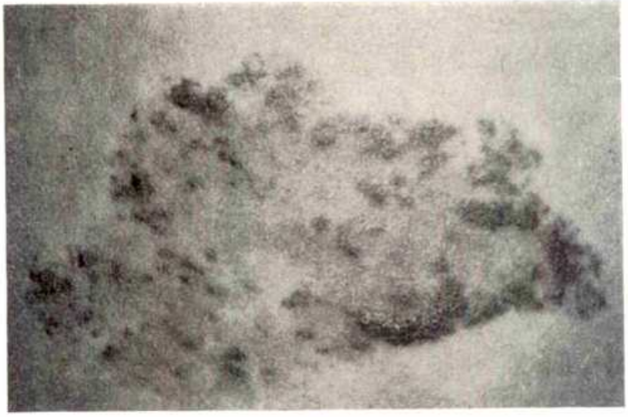
FIG. 4.—Case 2 at 25 days. Superficial strawberry mark.

Case 3.—A female infant born normally of a healthy mother. At 5 days a 13 × 23 mm, irregularly shaped, rose-coloured, flat, smooth erythema was observed on the inner aspect of the left lower leg. The erythema was surrounded by a 2 mm wide discrete pale halo. On close inspection this consisted of extremely fine telangiectasia (Fig. 5).

At 8 days the patch was still flat, but more distinct, and the pale halo had become more discrete. By 17 days the patch had become bright red, slightly raised, with a fine granular surface (superficial type of strawberry mark). The pale halo was hardly visible (Fig. 6). At 30 days the patch had risen more distinctly and had become dark red.

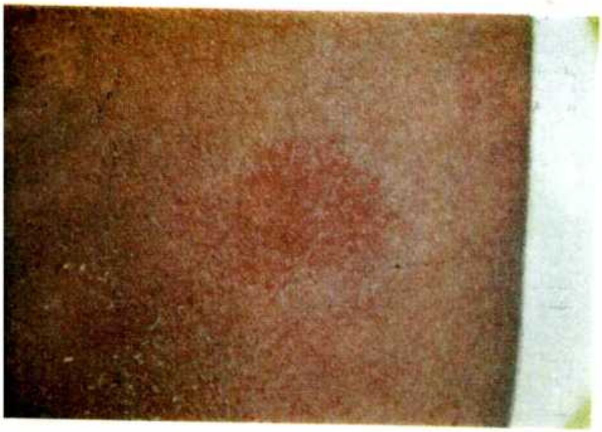

FIG. 5.—Case 3 at 5 days. Telangiectatic patch on the leg.

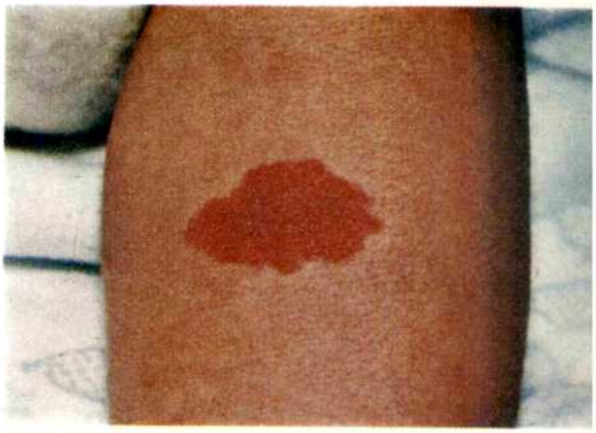

FIG. 6.—Case 3 at 17 days. Superficial strawberry mark.

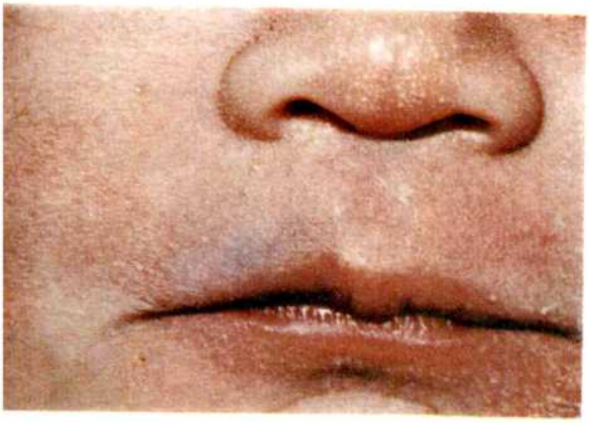

FIG. 7.—Case 4 on the day of birth. Bruise-like macule on the lip.

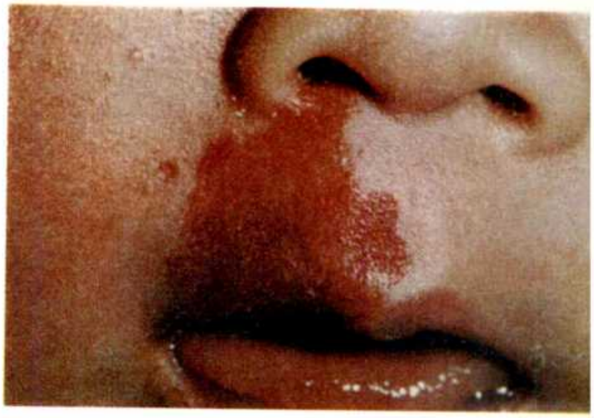

FIG. 8.—Case 4 at 18 days. The erythema is elevated and indurated.

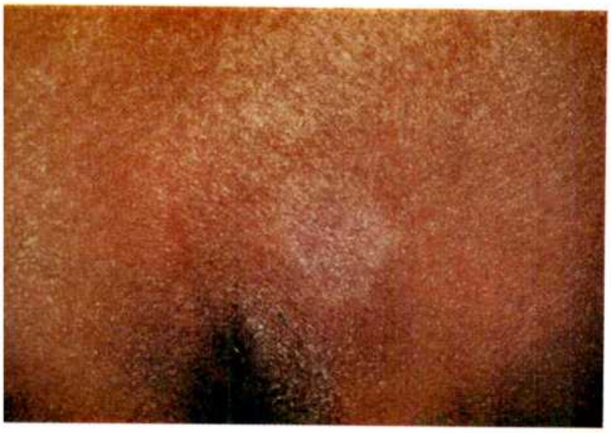

FIG. 9.—Case 5 at 2 days. Pale patch on the buttock.

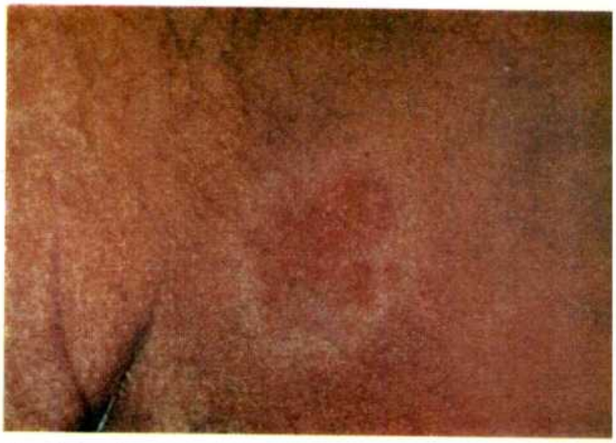

FIG. 10.—Case 5 at 25 days. Red papules appearing on the pale patch.

[To face p. 140]

At 45 days red papules were first noticed on the right wrist and on a toe. Following application of carbon dioxide snow, the angioma of the leg became barely visible after the age of 3 months.

Case 4.—A female infant born normally of a healthy mother. On the day of birth a bruise-like, bluish macule was noticed above the vermilion border of the right upper lip. There was no pallor, and the macule seemed on palpation to be somewhat infiltrated (Fig. 7). According to the mother, this patch became red by the age of 10 days. At 18 days a vivid red, slightly granular erythema was noticed on the right half of the upper lip. The mucosal portion of the upper lip was also involved (Fig. 8). By 25 days the patch had risen markedly and the upper lip had become swollen (mixed type of strawberry mark). There was an ulcer on the labial border. The blood count was normal, except for the platelets, which were 97,000/mm^{-3}. Dexamethasone was given orally in daily doses of 1·5 mg, and the swelling of the lip regressed in 2 weeks. Dexamethasone was then reduced in dosage, but the ulcer extended as far as the gum. As the upper lip was only slightly swollen and the ulcer had almost disappeared, oral steroids were stopped at 2 months. Thereafter, the swelling of the lip was at times pronounced; but, by 15 months, the redness and swelling were much reduced.

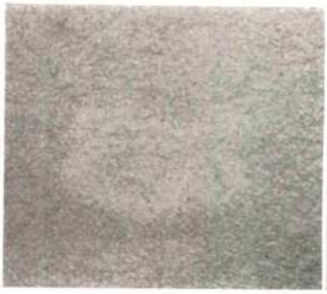

FIG. 11.—Case 6 at 3 days. Pale patch on the back.

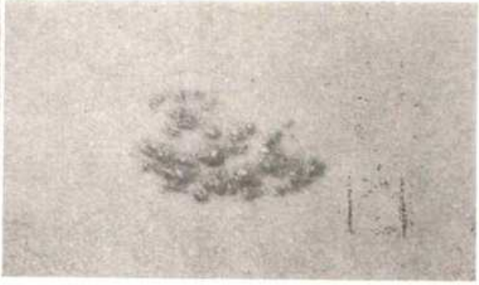

FIG. 12.—Case 6 at 55 days. Red papules scattered.

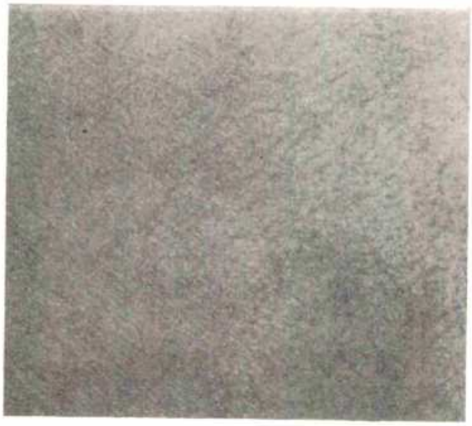

FIG. 13.—Case 7 at 1 day. Pale patch on the back.

FIG. 14.—Case 7 at 38 days.

Case 5.—A female infant born by Caesarian section of a healthy mother. At 2 days, a pale patch 10 mm square, was noticed on the right buttock. This patch seemed to be somewhat concave, but no resistance was palpable (Fig. 9). At 9 days, discrete telangiectasia appeared on this patch. At 25 days the patch remained pale and slightly concave, but pinhead-sized, bright red macules of erythema were grouped in the centre (Fig. 10). By 50 days the patch had become a raised, granular, red plaque surrounded by a discrete pale halo (superficial type). By 12 months the naevus had disappeared spontaneously, but a very discrete erythema and a pale halo were still visible. At 17 months of age only a slight pallor was noted.

Recently two similar cases have been observed.

Case 6.—A female infant born normally of a healthy mother. At 3 days a well defined, oval, pale patch 20 × 25 mm was noted on the back (Fig. 11). At 17 days irregular telangiectasia appeared on this patch, and, at 23 days, miliary erythema was noticed on the telangiectasia, while the local pallor was scarcely detectable. At 55 days vivid red papules were observed, scattered but partially coalescing on the initially pale area (Fig. 12).

Case 7.—A female infant born normally of a healthy mother. At 1 day, a well demarcated, rectangular, pale patch 15 × 20 mm was noted on the back (Fig. 13). At 6 days fine telangiectasia appeared on this patch. By 26 days the local pallor had become less marked, and flat macules of erythema, 1 mm in diameter, accompanied by telangiectasia, were observed. By 38 days the macules had coalesced and were slightly elevated, while the local pallor was hardly visible and the telangiectasia had disappeared (Fig. 14).

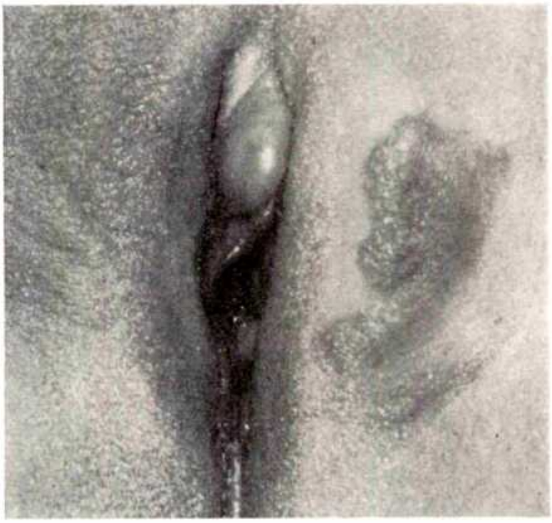

FIG. 15.—Case 8 at 11 weeks. Nodular strawberry mark on the vulva.

Case 8.—A female infant born by breech presentation of a healthy mother. On the 4th day no abnormality was found. On the 11th day a sharply demarcated, uniform erythema, 6 × 12 mm was noticed on the left labium majus. Neither a pale halo nor a telangiectatic pattern was visible at that time. At 23 days it remained a simple erythema, and the diagnosis of portwine stain was made. At 5 weeks it still seemed to be a portwine stain. At 8 weeks a slight elevation was first noticed, and it was at this time that the strawberry mark was suspected. At 11 weeks a nodular strawberry mark was observed (Fig. 15).

DISCUSSION

In many papers dealing with the natural history of angiomata, the account of the onset of the strawberry mark is very vague; most authors state that it begins at birth or soon after but give no precise description of the " angioma " at birth. Degos (1970) states that it is not exceptional to note a strawberry mark which at birth has the character of a portwine stain and becomes elevated during the first weeks or months. According to him, this is the atypical origin of the circumscribed, or superficial, *en plaque* type of strawberry mark. He stresses the difficulty of diagnosing these conditions from the portwine stain. Bowers *et al.* (1960)

noted that the strawberry mark is sometimes quite flat at onset, and that it may at first be mistaken for a portwine stain, or even a large spider telangiectasis.

Perlman (1960) recorded that the strawberry mark appears at times as a cluster of minute, closely packed, pinhead lesions which, after several weeks, attain the characteristic size and appearance. According to Schnyder (1966), the beginning of the strawberry mark consists of pinhead-sized red papules or an ill-defined flat erythema. Pratt (1953) examined 1096 infants of which the great majority were between 4 and 8 days of age. He noted that the strawberry marks on the white infants were bright ruby in colour, while those on the Negro infants were a darker, bluish red. A few lesions were small puncta, although the majority ranged from 0·5 to 2 cm in diameter. All but the smallest were distinctly elevated.

Nishiwaki (1968) described a case of strawberry mark which began as a telangiectatic patch. In a female infant a telangiectatic, violet-red, band-like zone was first noticed on the leg at the age of 3 days. At 8 days, the redness became more vivid, and the telangiectasia less distinct. At 29 days a typical plaque-like strawberry mark was observed.

The pale patch in our series is identical to the pale spot demonstrated by Payne *et al.* (1966) as a precursor to the haemangioma. In their 6 observations the first phase was a well demarcated, non-elevated, pale area, usually visible when the infant cried. The second phase was the appearance of small telangiectases scattered throughout the pale area. This phase was then followed by the appearance of pebbly haemangioma. Interestingly, one of their cases was arrested at the stage of telangiectasis and did not achieve the clinical features of a strawberry mark.

In our observations 3 strawberry marks began with telangiectasia surrounded by a pale halo, 3 began as a pale patch, one as a bruise-like macule, and one as simple erythema. On the pale patch, fine telangiectases appeared after several days as the second phase, as described by Payne *et al.* (1966). Our impression is that the usual pattern of the earliest strawberry mark is a non-raised, telangiectatic lesion or localized pallor. This local pallor disappears one to several months after birth; its nature is unknown. It is not certain that the telangiectatic patch of our series corresponds to the large spider telangiectasis described by Bowers *et al.* (1960) because a detailed description was not given.

The telangiectasia described above is thread-like and is much finer and more subtle than the superficial dilated vessels frequently noted on the cheeks or flanks of newborn infants. The differentiation between the pale patch and a naevus anaemicus is more difficult. The pale patch may be solitary and more or less oval. If such patches are segmented, systematized, or polygonal in shape, the diagnosis is probably naevus anaemicus.

The flat uniform erythema as noticed in our Case 8 was also described by Degos (1970), Schnyder (1966) and Bowers *et al.* (1960). The differential diagnosis between this feature of the strawberry mark and a portwine stain may at times be difficult. Generally, the portwine stain presents a more vivid and uniform redness and a more regular and sharp border. In Case 8 the erythema was absolutely flat and uniform, and neither telangiectasia nor a pale halo was noticed. For this reason the diagnosis of a portwine stain was made, and the diagnosis of strawberry mark was not established until the 8th week.

The closely packed pinhead lesions noted by Perlman (1960) and the red small papules described by Schnyder (1966) may not be the earliest sign of a growing angioma. In fact, the pinhead erythema and papules appeared mainly on the pre-existing telangiectasia. Moreover, in the newborn in their first week, a red punctum or a small red papule may simulate a toxic erythema or petechiae so frequently found in this period.

The bluish macule is not noted in previous reports, except for Holden and Alexander's (1970) second case, in which multiple deep bluish or purple subcutaneous papules and nodules were observed at birth. In this diffuse neonatal haemangiomatosis, the course of the bluish elements could not be established, since the infant died soon after.

In our series of 2168 infants no strawberry mark was noticed as a raised lesion within the first 2 weeks of life, but was found to appear principally between the third and fifth week. In the 5 cases of Payne *et al.* (1966) the time of appearance of the haemangioma was given as 1 year, 4 months, 10 weeks, 2 months and 2 weeks respectively. These findings are not in accordance with the experience of Pratt (1953) who observed distinctly elevated angiomata within 8 days of life. The onset of the strawberry mark has been described as " at birth " by most authors on the basis of the mothers' statements. There is, however, sufficient evidence that the true strawberry mark makes its appearance later in most cases. We would like to define the onset of the angioma as the time of the first elevation. The early changes described above do not yet constitute strawberry marks, but may be regarded as precursors. In fact, the pale patch can remain an abortive angioma: conversely, the strawberry mark can appear on the apparently normal skin.

Nevertheless, from findings such as telangiectasia or localized pallor, the diagnosis of a suspected strawberry mark can be put forward at the time when the newborn is discharged from hospital.

We are indebted to the Daiichi Pharmaceutical Co. Ltd for providing the colour plates.

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PROGNOSIS IN INDUSTRIAL DERMATITIS

D. BURROWS

Summary.—One hundred and thirteen patients with industrial dermatitis were reviewed 10–13 years later. Seventy-nine per cent still required treatment from time to time. Cement dermatitis had a significantly worse prognosis than oil dermatitis. Only 8% of cases of cement dermatitis were clear.

In a review of cases for which industrial injuries benefit is paid in Northern Ireland there were about 150 successful claims for contact dermatitis per year, this being about 1% of all industrial injuries occurring in a working population of 500,000.

The incidence of dermatitis diminishes with age, but the risk has been reduced by only about 50% in the older age group 55–64 compared with the younger age group 25–34.

Information about, and the factors influencing, the long-term prognosis in industrial dermatitis are important because industrial dermatitis is probably responsible for more morbidity and lost days in industry than is generally realized. Yet very little is known about the incidence and prognosis of this condition (*Br. med. J.*, 1971). It is important to be able to advise about future employment and treatment, and to know whether the way of life and standard of living have been impaired, and if any compensation is adequate to meet this.

Hellier (1958) found that 77% of cases he examined for the Ministry of National Insurance still had trouble with their skin after 4 to 6 years. In Mayr's (1938) series only 32% of cases of contact dermatitis still had trouble after 10 years. More recently Johnston and Wilson (1971), in a study of cases of contact dermatitis to oil seen for medico-legal reports, found 40 of 100 cases still having trouble after 6 to 13 years.

Size of Problem

In Northern Ireland, with a working population in the region of 500,000 (total population 1,500,000), there are about 150 successful claims for industrial injuries benefit for contact dermatitis per year, making about 1% of all industrial injuries. Because a person is off work for a longer period with dermatitis than with the average injury, the number of days lost through industrial dermatitis (20,171 days —1969/70) is 5% of the total days lost through all industrial injuries (453,582 days—1969/70). This can be compared with 118,900 days lost through strikes in the same period. All these cases are severe enough to require a spell of absence from work. The total number of cases of industrial dermatitis must be higher, as some are mild enough not to need to stop work and simply require a change of job.

There are about 52,000 people employed in the building and construction industry in Northern Ireland and, allowing an average working life of 50 years in

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industry and unchanged working conditions, it may be calculated that about 3·5% of people working in the building industry will develop a contact dermatitis severe enough to warrant a spell off work. Industries associated with rubber manufacture employ about 7000 people and, on the same basis, a man would have about a 10% chance of developing dermatitis if he remained in this industry. Obviously, in both these industries, there are some occupations which carry a much higher risk than others.

TABLE I.—*Cause of Dermatitis in Cases Being Paid Industrial Injuries Benefit in Northern Ireland*—1969/70

Type of irritant	Male (%)	Female (%)
Cement/sand/mortar	23	Nil
Detergents/cleaning fluids	Nil	13
Rash following laceration	1	Nil
Rubber	9	1
Oil	18	8
Chrome	3	Nil
Fibre (natural/synthetic)	1	4
Chemicals (including dyes and plastics)	10	4
Flour	1	1
Food preparation	Nil	2
Tobacco manufacture	1	Nil
	67	33

TABLE II.—*Industrial Dermatitis Injuries Benefit Analysed by Age Group*

	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64
Percentage of population in age group	8·4	7·4	6·2	5·6	5·4	5·5	5·7	5·2	5·2	4·7
Male cases of industrial dermatitis % of male total	10·8	12·7	13·3	12·0	11·4	12·0	10·1	7·7	5·7	4·4
Female cases of industrial dermatitis % of female total	18·5	16·9	9·2	7·7	12·3	12·3	15·3	3·0	4·6	

The figures for women (Table II) were considered separately because they are directly influenced by marriage, leaving work and returning subsequently to work when the family is older. The percentage (men) for the 15–24 year group is probably a little low due to quite a large percentage of the population still being in full-time education. These figures confirm that the risk of dermatitis diminishes with increasing age, but the risk of contact dermatitis in the older worker is still high. The incidence in the age group 24–35 is only just over twice that in the age group 55–64.

INVESTIGATION

One hundred and eighty-four patients who had been seen 10–13 years previously for medico-legal reports were reviewed. These patients were seen either by the late Dr J. Jefferson or the author. At the original examination very full notes were made of the patients' total employment, medical history and rash. Thirty patients were discarded on review as the original diagnosis of contact dermatitis seemed questionable. Seven had died and it was not possible to trace 34. Letters were sent to the patients asking them to attend for re-examination; failing this a medical social worker visited the addresses and tried to trace and interview the patients.

There are several difficulties in any long-term follow-up of industrial dermatitis. It is often only the severest cases who are seen by the dermatologist and thoroughly investigated. Certainly it is likely to be the severer cases who will be seen for medico-legal reports. Patients are difficult to trace after a long interval, and in this series more than an average number lived in areas which have been redeveloped, which increased the difficulty of contacting them. Very few patients had done no work of any sort again; a person was considered " not working " if he was not able to work more than 6 weeks per year because of his skin. It is difficult, because of the increase in the cost of living and rise in wages, to assess whether the patient has sustained any real loss. Furthermore, it is impossible to forecast a person's future earnings. Some move up the earning scale, but some earn less as they grow older, particularly in those occupations demanding physical strength. Awards given in the courts were taken into consideration. In 3 cases it was impossible to make any estimation of the patient's financial state. Those who had had no trouble with their skin in the preceding 4 years were considered clear. All others, even if they had only occasional trouble, were included in the " not clear " group. The " not clear " group was further subdivided into those who were " bad " and others.

TABLE III.—*Prognosis*

	Under 40	Over 40	Total
Not working	7	18	25
Working	44	44	88
Loss	32	32	64
No loss	19	27	46
Clear	11	13	24
Not clear	40	49	89
Bad	22	22	44

TABLE IV.—*Cases Sub-divided into Occupational Sub-groups*

	Cement	Oil	Fibre-glass	Baker	Oil folliculitis	Epoxy-resin	Deter-gent	Miscel-laneous	Total
Not working									
(under 40)	3	1	0	0	0	0	1	2	7
(over 40)	6	4	0	0	0	0	3	5	18
Working									
(under 40)	7	10	3	4	4	4	1	11	44
(over 40)	9	16	3	2	2	2	1	9	44
Loss									
(under 40)	8	5	2	4	1	4	1	7	32
(over 40)	9	9	0	0	1	2	3	8	32
No loss									
(under 40)	2	6	1	0	3	0	1	6	19
(over 40)	5	10	3	2	1	0	1	5	27
Clear									
(under 40)	0	3	1	0	1	1	0	5	11
(over 40)	2	4	1	0	0	0	2	4	13
Not clear									
(under 40)	10	8	2	4	3	3	2	8	40
(over 40)	13	15	3	2	2	2	2	10	49
Bad									
(under 40)	6	2	3	3	1	1	2	4	22
(over 40)	8	5	1	1	0	0	0	7	22

DISCUSSION

There was no statistically significant difference between people who developed dermatitis under or over the age of 40, in regard to future work prospects, prognosis, or financial state, although the work/not working group only just failed to make conventional levels of significance ($0{\cdot}10 > P > 0{\cdot}05$). It is worth noting, however, that only 14% of those who developed dermatitis before 40 were not working, whereas 29% of those who developed skin trouble after 40 had not worked to any great degree. The reason for this is the obviously greater incentive to younger men to work, their greater ability to learn a new trade and their general adaptability.

The figure of 79% of patients still having trouble with their skin compares closely with Hellier's figure of 77% having trouble in the shorter period of 3 to 4 years. Quite a number who were still having trouble with their skin were in useful employment and had found a job which suited their skin. There were, however, quite a number who were not working but whose skin was clear. This was for several reasons. Many had no training and had worked only as builders or labourers, and either this was the only type of work available in their area or firms would not employ a man with a history of dermatitis. Many felt that any physical work worsened their skin condition and were unable to obtain a clean, dry job. It is therefore not surprising, in a region where 8% unemployment allows employers to be selective, to find that 22% were not working.

Too much weight cannot be placed on the "loss/no loss" figures, for here assessment cannot be exact. Who can say what a man might have done had he not developed dermatitis? The great majority felt even with their claim in common law they had suffered definite financial loss. There is no doubt a strong case for some reappraisal of the industrial injuries system, which should take a longer view in cases of industrial dermatitis.

The numbers in the various occupational sub-groups were small, which made statistical analysis difficult. The difference in prognosis between the cement group *versus* the rest was not "statistically" significant at conventional levels $P > 0{\cdot}05$, but it could be considered of some "clinical" significance that only 8% of the cement group were classified as "clear" compared to 25% of the rest. These figures compare closely with the earlier findings of Burrows and Calnan (1965), who found that only 8% of cases of cement dermatitis were clear 5 months later. The prognosis in cement dermatitis appears not to improve with time.

Johnston and Wilson (1971) found a better prognosis in their oil dermatitis group (60% clear) than in Hellier's heterogeneous group (22% clear). In this series, when the "Cement" group was compared with the "Oil" group, the prognosis was significantly better in the oil group ($P = 0{\cdot}0396$). In the oil dermatitis group 77% were clear compared with 8% in the cement dermatitis group. It may be that direct irritant dermatitis has a better prognosis than sensitization dermatitis.

My thanks are due to Miss S. Cooke, Medical Social Worker, for her work in tracing these patients and to Dr T. J. Hagan, Senior Medical Officer, Ministry of Health and Social Services, for the information about industrial injuries. My thanks are due also to the Northern Ireland Hospitals Authority for a grant to carry out this survey.

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THE EFFECT OF CHRONIC SUN DAMAGE ON THE ACTIVITY OF METABOLIC ENZYMES OF THE EPIDERMIS OF HUMAN SKIN*

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Summary.—The activity of 14 enzymes representative of the main metabolic pathways was assayed with microfluorometric techniques in the epidermis of exposed and unexposed skin of old men.

The activity of 9 enzymes (α-glycero-phosphate dehydrogenase, enolase, lactic dehydrogenase, isocitric and malic dehydrogenase, glucose-6-phosphate dehydrogenase, alanine and aspartate transaminase, β-hydroxylacyl dehydrogenase) was decreased in the exposed skin of old humans.

The decrease of the activity of many enzymes in the exposed skin seems to be due to the accumulation of damage to epidermal cells.

Chronic sun damage induces many clinical, morphological and biochemical alterations in the skin which recently have been thoroughly reviewed (Cerimele and Serri, 1972).

The great biological importance of chronic sun damage depends on the fact that the frequency of neoplasms on the skin of the areas usually exposed to the sun's rays (e.g. face, back of hands) is 100 times higher than on the skin of unexposed areas (e.g. abdomen): this leads us to regard chronic sun damage as a praecancerous state.

Some interesting alterations observed in the activity of metabolic enzymes of the epidermis of Prosimian skin treated with chemical carcinogens (Adachi *et al.*, 1969) prompted us to study these enzymes in the epidermis of exposed and unexposed areas of old men.

The present work was undertaken to determine whether any enzymatic alteration could be detected in chronically sun-exposed skin as compared with unexposed skin.

MATERIALS AND METHODS

Two groups, each of 5 healthy human volunteers, were studied. The age of the first group, which we will call " young ", was in the range 20–25 years: 4 were males, one was female, and all were students. The age of the second group, which we will call " old " was in the range 76–86 years: 3 were males, 2 were females, and all were farmers who had spent a large part of their life in open air exposed to the sun.

The " old " group was chosen to investigate the effect of prolonged exposure to solar radiation, while the " young " group was used as control, and to check the possible regional differences between the skin of the back of the hands and the skin of the abdomen.

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From each subject 2 skin biopsies were obtained with an 8 mm electric punch, one from the lower left part of the abdomen (this will be referred to as " unexposed skin "), the other from the back of the left hand (this will be referred to as " exposed skin "). The clinical and histological appearances of the unexposed skin were not very different between the young and the old groups, whereas great differences were observed in the exposed skin. The skin of the subjects of the old group showed the clinical features of the chronic sun-damaged, " farmer's skin ", and the histological control showed typical " solar degeneration ".

The biopsies were immediately frozen in solid carbon dioxide and then cut 20 μm in thickness in a cryostat at -20°C. The frozen sections were dried in a vacuum to preserve the activity of the enzymes; the epidermis was dissected and small samples (0·3–1 μg) weighed with a quartz fishpole balance and transferred into small test tubes according to Lowry's method (1953).

Fourteen enzymes representative of the glycolytic pathway, the tricarboxylic acid cycle, the transamination linkages between amino-acid and carbohydrate metabolism, the pentose phosphate pathway and fatty acid metabolism have been studied. The enzymes' activities were assayed by microfluorometric methods (Lowry *et al.*, 1957), as adapted to the skin by Hershey *et al.* (1960).

The detailed methods for the single enzymes have been described by Adachi (1967).

The following enzymes have been assayed: (1) hexokinase; (2) phosphofructokinase; (3) α-glycerophosphate dehydrogenase; (4) glyceraldehyde-3-phosphate dehydrogenase; (5) enolase; (6) lactic dehydrogenase (LDH); (7) isocitric dehydrogenase (ICDH); (8) malic dehydrogenase (MDH); (9) malic enzyme (ME); (10) alanine transaminase; (11) aspartate transaminase; (12) glucose-6-phosphate-dehydrogenase (G-6-PDH); (13) 6-phosphogluconic-dehydrogenase (6-PGDH); (14) β-hydroxylacyl-dehydrogenase (B-HO-DH).

RESULTS

In the group of young people, on comparing the epidermis of exposed skin with the epidermis of unexposed skin, higher activity of the following enzymes has been observed in the former: phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, enolase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, aspartate transaminase (Table I). The enhancement of the activity

Table I.—*Enzyme Activities in the Epidermis of Skin of Young Humans*

Enzymes	Unexposed (means ± SE)	Exposed (means ± SE)	*t*	*P*
Hexokinase	0·691 ± 0·064	0·786 ± 0·022	−1·668	—
Phosphofructokinase	0·706 ± 0·144	0·903 ± 0·157	−6·207	< 0·01
α-Glycerophosphate-dehdryogenase	0·159 ± 0·016	0·155 ± 0·008	0·299	—
Glyceraldehyde-3-phosphate dehydrogenase	6·457 ± 0·685	8·536 ± 1·073	−3·361	< 0·05
Enolase	10·016 ± 0·210	15·582 ± 0·806	−8·369	< 0·01
Lactic dehydrogenase	34·640 ± 1·666	36·820 ± 2·197	−1·206	—
Isocitric dehydrogenase	1·719 ± 0·155	1·603 ± 0·187	1·311	—
Malic dehydrogenase	32·176 ± 2·819	33·304 ± 2·787	−1·335	—
Malic enzyme	0·184 ± 0·014	0·171 ± 0·025	0·583	—
Glucose-6-phosphate dehydrogenase	0·729 ± 0·092	1·180 ± 0·101	−7·157	< 0·01
6-Phosphogluconic dehydrogenase	0·305 ± 0·041	0·500 ± 0·039	−5·654	< 0·01
Alanine transaminase	1·128 ± 0·131	0·941 ± 0·075	1·823	—
Aspartate transaminase	2·642 ± 0·100	3·129 ± 0·128	−7·104	< 0·01
β-Hydroxylacyl dehydrogenase	2·331 ± 0·205	2·342 ± 0·089	−0·055	—

Each enzyme activity is expressed as mol/kg dry weight/hour. The data expressed in the table are the means of 5 subjects: the single data of each biopsy are the means of 5 determinations.

of glyceraldehyde-3-phosphate dehydrogenase is statistically significant ($P<0\cdot05$); the enhancement of all other enzymes is highly significant ($P < 0\cdot01$). The metabolic pathways affected by the regional difference are the glycolytic pathway (phosphofructokinase, glyceraldehyde-3-phosphate dehydrogenase, enolase) and the pentose-phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase) where the activity of the enzymes is stimulated.

In the group of old people, in the epidermis of exposed areas compared with the epidermis of unexposed areas, the activity of enolase, glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase was still enhanced, while the activity of α-glycerophosphate dehydrogenase, isocitric dehydrogenase, malic

TABLE II.—*Enzyme Activities in the Epidermis of Skin in Old Humans*

Enzymes	Unexposed A(means ± SE)	Exposed B(means ± SE)	*t*	*P*
Hexokinase	0·700 ± 0·064	0·733 ± 0·057	−1·369	—
Phosphofructokinase	1·134 ± 0·149	0·948 ± 0·083	+1·583	—
α-Glycerophosphate-dehydrogenase	0·142 ± 0·006	0·101 ± 0·015	+3·684	< 0·05
Glyceraldehyde-3-phosphate dehydrogenase	6·022 ± 0·202	6·518 ± 0·543	−1·206	—
Enolase	7·704 ± 0·830	9·344 ± 1·200	−3·265	< 0·05
Lactic dehydrogenase	27·096 ± 1·341	23·162 ± 1·403	+1·516	—
Isocitric dehydrogenase	1·330 ± 0·114	1·068 ± 0·080	+3·941	< 0·05
Malic dehydrogenase	20·874 ± 2·729	20·958 ± 2·923	−0·096	—
Malic enzyme	0·169 ± 0·023	0·135 ± 0·029	+3·696	< 0·05
Glucose-6-phosphate dehydrogenase	0·557 ± 0·037	0·772 ± 0·075	−3·359	< 0·05
6-Phosphogluconic dehydrogenase	0·256 ± 0·035	0·376 ± 0·071	−2·896	< 0·05
Alanine transaminase	0·982 ± 0·057	0·515 ± 0·047	+13·843	< 0·01
Aspartate transaminase	2·300 ± 0·206	2·012 ± 0·232	+1·800	—
β-Hydroxylacil-dehydrogenase	1·966 ± 0·244	1·618 ± 0·205	+6·121	< 0·01

Each enzyme activity is expressed as mol/kg dry weight/hour. The data expressed in the table are the means of 5 subjects: the single data of each biopsy are the means of 5 determinations.

TABLE III.—*Enzyme Activities in the Epidermis of Skin of Young and Old Humans*

Enzymes	Unexposed			Exposed		
	Young	Old	*t*	Young	Old	*t*
Hexokinase	0·691	0·700	0·106	0·786	0·733	0·887
Phosphofructokinase	0·706	1·134	2·062	0·903	0·948	0·252
α-Glycerophosphate-dehydrogenase	0·159	0·142	1·027	0·155	0·101	3·231*
Glyceraldehyde-3-phosphate dehydrogenase	6·457	6·022	0·609	8·536	6·518	1·713
Enolase	10·016	7·704	2·700*	15·582	9·344	4·319†
Lactic dehydrogenase	34·640	27·096	3·528†	36·820	23·162	5·241†
Isocitric dehydrogenase	1·719	1·330	2·022	1·603	1·068	2·656*
Malic dehydrogenase	32·176	20·874	2·881*	33·304	20·958	3·057*
Malic enzyme	0·184	0·169	0·564	0·171	0·135	1·000
Glucose-6-phosphate dehydrogenase	0·729	0·557	1·725	1·180	0·772	3·249*
6-Phosphogluconic dehydrogenase	0·305	0·256	0·896	0·500	0·376	1·543
Alanine transaminase	1·128	0·982	1·026	0·941	0·515	4·784†
Aspartate transaminase	2·642	2·300	1·496	3·129	2·012	4·218†
β-Hydroxylacil-dehydrogenase	2·331	1·966	1·146	2·342	1·618	3·248

Each enzyme activity is expressed as mol/kg dry weight/hour. The data expressed in the table are the means of 5 subjects: the single data of each biopsy are the means of 5 determinations.

* $P<0\cdot05$; † $P<0\cdot01$.

enzyme, alanine transaminase and β-hydroxylacyl-dehydrogenase was depressed (Table II). The diminution of the activity of α-glycerophosphate dehydrogenase, isocitric dehydrogenase and malic enzyme is statistically significant ($P < 0{\cdot}05$); the diminution of alanine-transaminase and β-hydroxyacyl-dehydrogenase is highly significant ($P < 0{\cdot}01$).

The comparison between the results in exposed skin of the young and old groups showed that the activity of almost all the enzymes was depressed in the epidermis of the old group: phosphofructokinase was the only exception, but was statistically not significant (Table III).

The decrease of α-glycerophosphate dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, glucose-6-phosphate dehydrogenase and β-hydroxylacyl-dehydrogenase was statistically significant ($P < 0{\cdot}05$); the decrease of enolase, lactic dehydrogenase, alanine transaminase and aspartate transaminase was highly significant ($P < 0{\cdot}01$).

Expressing the results as percentual ratios (Table IV), it is clear that the most interesting results are shown in the third column, where the activity of the enzymes in the exposed skin of young and old people is compared: 13 of 14 enzymes studied are decreased in exposed skin of old people.

TABLE IV.—*Difference of the Activity of* 14 *Enzymes between the Epidermis of the Exposed and Unexposed Areas in Young People* (*First Column*), *Old People* (*Second Column*) *and between the Exposed Areas of Old and Young People* (*Third Column*). *The Values are Expressed as Percentual Variations*

Enzymes	Young (%)	Old (%)	Exposed (%)
Hexokinase	+13·7	+ 4·7	− 6·7
Phosphofructokinase	+27·9	−16·4	+ 5·0
α-Glycerophosphate dehydrogenase	− 2·5	−28·9	−35·0
Glyceraldehyde-3-phosphate dehydrogenase	+32·1	+ 8·2	−23·6
Enolase	+55·5	+21·2	−40·0
Lactic dehydrogenase	+ 6·2	−14·5	−42·5
Isocitric dehydrogenase	− 6·7	−19·6	−33·3
Malic dehydrogenase	+ 3·5	+ 0·402	−37·0
Malic enzyme	− 7·0	−20·1	−21·0
Glucose-6-phosphate dehydrogenase	+62·0	+38·6	−34·5
6-Phosphogluconic dehydrogenase	+64·0	+47·0	−24·8
Alanine transaminase	−16·5	−47·5	−45·2
Aspartate transaminase	+18·5	−12·5	−35·6
β-Hydroxylacil-dehydrogenase	+ 0·472	−17·7	−31·0

DISCUSSION

The only previous paper concerned with the activity of metabolic enzymes in relation to ageing and regional variations in man reported that the activity of many enzymes was depressed in the aged skin, but failed to report significant variations in relation to the anatomical region of the skin (Salfeld, 1966).

We have obtained rather different results. In a study of the skin of the abdomen of 63 subjects ranging in age from 9 months throughout life to 90 years, we were unable to find any significant difference in the activity of the enzymes studied (Yamasawa *et al.*, 1972), while in the present work some specific alterations could be shown to be related to chronic sun damage.

Some conclusions can be drawn from the critical consideration of our results. First there is a common trend towards a lowering of the activity of many enzymes in the chronically sun-damaged skin; this lowering affects in different degree the different metabolic pathways.

Both transaminases are highly depressed; the enzymes of the glycolytic pathway, the Krebs cycle and the fatty acid metabolism show a smaller depression, while the enzymes of the pentose phosphate shunt are only slightly affected.

This last result could be explained by the fact that, even in old age, the epidermal cells show high mitotic activity and therefore have a high requirement of pentose sugars which are produced through the pentose phosphate pathway.

The depression of the activity of many enzymes in chronically sun-exposed skin could be due to the accumulation of damage to the epidermal cells, more than to some selective alteration. These alterations are not easily related to those observed in carcinogen-induced hyperplasia (Adachi *et al.*, 1969) and in human tumours (Cerimele *et al.*, 1971) where enhancement of enzyme activity is usually observed.

Our results could fit with the hypothesis that the chronic sun damage of the human skin, and the neoplasms that develop on such skin, are two related but different processes. The chronic sun damage could be considered a degenerative process, and the decreased activity of some enzymes may be in agreement with this interpretation, while the development of skin tumours could be a different event. What actually triggers the transformation of a damaged epithelium into an anaplastic and metabolically active epithelium, as observed in skin tumours, is a problem still open to discussion.

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CONDITIONING OF SWEATING

A PRELIMINARY REPORT

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Summary.—Some characteristics of thermal sweating on the forearm and spontaneous sweating on the hand palm are described.

Instrumental conditioning of palmar sweating is reported.

THE literature contains a number of references to the possibility of modifying autonomically controlled visceral and glandular activities by classical or instrumental conditioning (Miller, 1969; DiCara, 1970). Conditioning of the galvanic skin reflex (GSR), which is thought to be some function of blood vascular or sweat gland activity (McLeary, 1965; Neumann and Blanton, 1970; Darrow and Gullickson, 1970), has also been described (Van Twyer and Kimmel, 1966; Randall and Sanford, 1971). From either point of view it is interesting to consider the possibility of conditioning sweat gland activity *per se*. The difficulty up till now has been the lack of automated methods for measuring sweat gland activity directly and sufficiently rapidly to be able to use the measurements themselves to operate some conditioning stimulus. There are now several devices suitable for this purpose (Spruit and Malten, 1966; Thiele and Malten, 1972).

The possibility of conditioning sweating is interesting both from the academic and clinical points of view; academically, since if conditioning of sweating produces conditioning of GSR, and vice versa, this will give further insight into the nature of the GSR; and clinically since it (a) might provide a therapeutic tool for dermatological patients with hyper- or hypo-hidrosis, and (b) could demonstrate, at least the possibility of, psychosomatic factors in some conditions involving abnormal sweat gland activity.

With these considerations in mind we have conducted a number of preliminary investigations into the physiological background of these problems and into some instrumental conditioning situations.

MATERIALS AND METHODS

Sweat measurements.—The sweat measuring device was the Meeco water vapour analyser, used in the fashion described by Spruit and Malten (1966).

This system uses a flow of dry nitrogen gas through a small metal cup (0·2 cm^2) placed on the skin surface. The water evaporating from the skin is carried in the nitrogen gas to the electrolytic cell of the Meeco. The water electrolyses H_2SO_4 in the cell and the resulting potential difference is recorded directly on a mV recorder.

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Stimulator.—A stimulator was designed and developed in these laboratories and the electronics workshop of the Medical School of the University of Nijmegen. The circuit is shown in Fig. 1 and the block diagram of the whole apparatus in Fig. 2.

Experimental

Range of normal subjects.—(a) The thermal sweat responses of 8 normal, male, paid volunteers were recorded. The subjects were seated, naked, in a dry sauna, and the measuring cup of the Meeco was placed on the inner surface of the arm, just below the elbow. The arm was supported on a stand inside the sauna. The water loss from the skin was recorded in this situation for some 10 min, after which the sauna was turned on. The water loss was recorded for 15 min, and the sauna was then turned off and the recordings continued for a further 10 min. This procedure was repeated on 2 subjects each day for 5 days. Skin and atmospheric temperatures were recorded simultaneously.

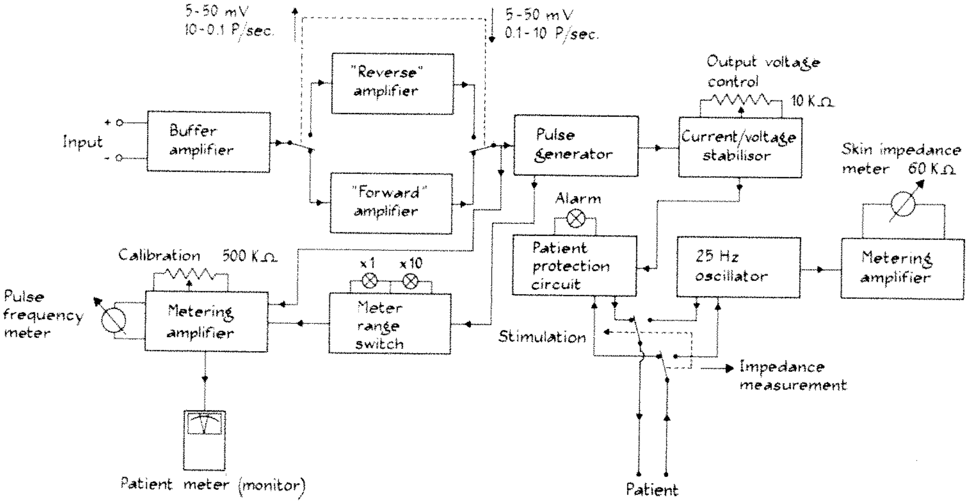

FIG. 1.—Schematic circuit of the shock apparatus.

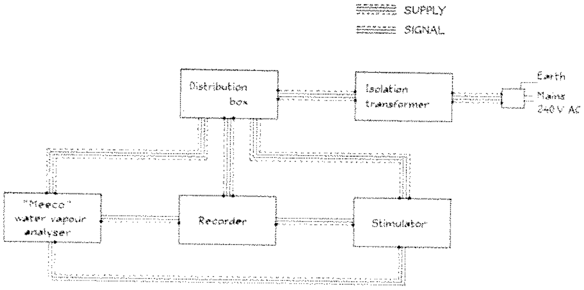

FIG. 2.—Block diagram of the measuring and stimulating system.

(b) The spontaneous water losses from the palm of the hand were recorded from 8 male volunteers by seating the subjects comfortably in a reclining chair and placing the measuring cup on the heel of the hand at the base of the thumb. Skin and atmospheric temperatures were recorded.

Conditioning experiments.—(a) A device was developed in the electronics department that would feed-back the output of the Meeco as a continuously variable frequency of electric shocks, the voltage of which was fixed and could be pre-set. Thus we could produce a situation in which increasing sweating could be increasingly painful, and decreasing sweating could be decreasingly painful (in terms of shock frequency). The level of the voltage was selected by slowly increasing the voltage until the subject could feel the shocks clearly. It was then maintained at that voltage for the rest of that trial.

A series of recordings were made without shocks, and then repeated with the shock apparatus switched on. The recordings from the Meeco were integrated for total volume of sweat produced per 40 min and plotted.

(b) A different conditioning method was later employed. In these experiments the sweating from the palm was recorded for some minutes without shocks, then for 10 min with the stimulator in circuit, and again for some minutes without stimulation. The stimulation was applied in the following way: if at the beginning of the stimulation period the subject's sweating was between 70 and 80% on the recorder scale, he was given one shock if he subsequently rose above 80%. When the value dropped below 70%, the subject was then given one shock if he subsequently rose above 70%. This was continued for the whole stimulation period. In each experiment the subject was studied for 3 periods with a chance of receiving shocks, and 4 periods with no chance of receiving shocks. This was then repeated on the following day and for one subject (No. 3) on the third day also.

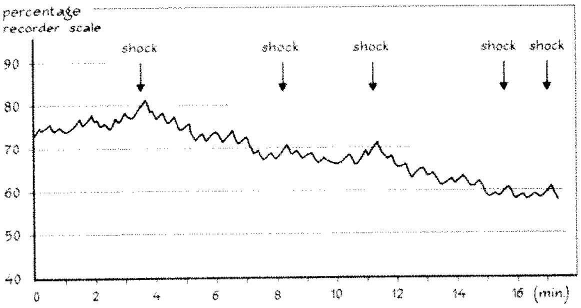

Fig. 3.—Diagrammatic representation of the conditioning procedure. For description see *Experimental: Conditioning experiments* (b).

Whatever the response of the subject, he was only ever given one shock in any one min period. The system is shown diagramatically in Fig. 3. With this system it was thus theoretically possible for the subject to avoid shocks altogether. Three subjects were studied in a total of 21 trials with this technique. One of the subjects knew the purpose of the experiments (Subject 1), and the other 2 were naive.

RESULTS

(a) *Responses of normal subjects to thermal stress.*—The subjects showed a wide range of water losses from immediate active sweating, even before the sauna was turned on, to no active sweating at the measuring site, even when the sauna reached 40°C. In all cases where sweating occurred a series of superimposed rhythms could be seen in the sweat response. The patterns were very complicated and the rhythms not always clear, since they all had very variable amplitudes. Small fluctuations of about 6/min and larger fluctuations of about 1/10 min were generally clearly seen; very slow fluctuations of about 1/45 min were also often visible. It seemed that the rhythms were real and omnipresent phenomena and that the total amount of sweat produced depended upon changes in the peak heights and not in the rhythms themselves. Generally, when the rhythms disappeared on lowering the environmental temperature, it seemed as though a threshold was passed; that is, it appeared that sweating was superimposed upon some characteristic physiological frequency. The types of sweat pattern seen are illustrated in Fig. 4.

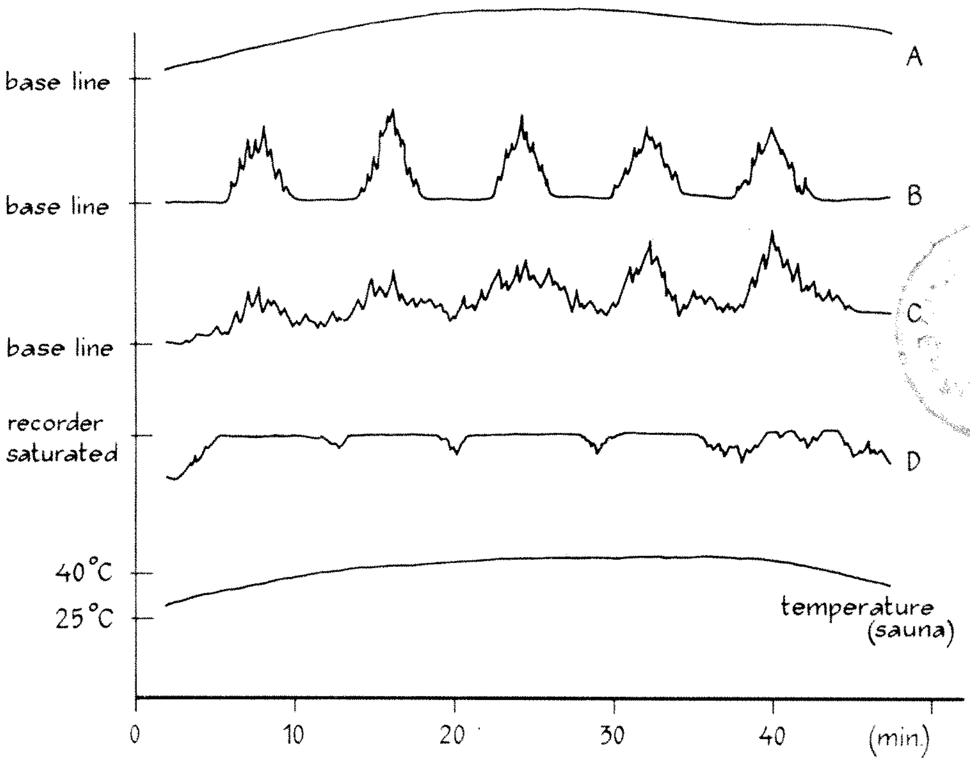

Fig. 4.—Diagrammatic representation of the various types of thermal sweating observed. These illustrations are not drawn to scale and indicate only the qualitative differences observed.

(b) *Spontaneous (non-thermal) sweating from the palm.*—This appeared to have the same series of frequencies as described in section (a) above. Thus both types of sweating were pulsatile.

The difficulties in analysis of these rhythms do not allow us to say whether they are universal or characteristic for an individual, or even if they are exactly the same as in thermal sweating; but they are, at least, very similar.

Conditioning.—(a) The results of these experiments were negative. The values for one subject are shown in Fig. 5.

(b) On the first trial with this system no response to the stimulus was seen. On the second, third and fourth trials Subject 1 stopped sweating soon after the onset of the stimulation period and began again only some few minutes after the stimulation period was over. In the first trial this subject received a shock each minute for the whole period. In the second and third trials he received 3 shocks in the whole trial period, and in the fourth trial he avoided receiving any shocks. The analysis of the 21 trials with the 3 subjects is shown in Table I.

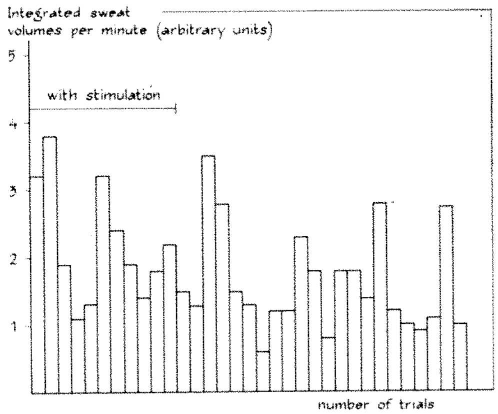

Fig. 5.—The sweat rates observed in one subject, using the variable shock frequency conditioning system. For description see *Experimental: Conditioning experiments* (a).

Table I.—*Comparison of the Sweat Rates from 3 Subjects under the Conditioning Situation Shown in Fig. 3*

Subject	Non-stimulation	Stimulation	Non-stimulation	Stimulation	Non-stimulation	Stimulation	Non-stimulation	Averaged stimulation, Previous non-st.	Averaged stimulation, following non-st.
1	1·135	0·473	0·497	0·604	0·703	0·430	0·561	75·3	96·5
1	0·561	0·443	0·600	0·338	0·380	0·324	—	73·9	81·4
2	1·430	1·420	1·440	1·180	1·290	0·830	1·160	81·7	86·9
2	1·340	1·200	1·090	1·150	1·050	0·880	0·790	92·8	110·0
3	1·690	0·685	0·524	0·422	0·619	0·498	0·686	67·1	90·3
3	0·862	0·558	0·593	0·391	0·413	0·305	0·340	68·2	92·8
3	0·613	0·461	0·418	0·333	0·557	0·417	0·352	79·7	96·2
Total average								76·8	90·4

Each "stimulation" period is expressed as a percentage of the preceding "non-stimulation" period (column 9) and as a percentage of the following "non-stimulation" period (column 10).

DISCUSSION

Theoretically, human sweating on the general body surface, which is temperature dependent, should be amenable to classical conditioning techniques, and the sweating on the palms, which is spontaneous, relatively temperature independent and often termed "emotional sweating", should be amenable to instrumental conditioning techniques. In classical conditioning one knows the stimulus that produces the response (the "unconditioned stimulus") and links this to another stimulus that has no previous significance for the response in the subject ("conditioned stimulus"). The best known version of this is Pavlov's original use of a bell (" conditioned stimulus "), food (" unconditioned stimulus ") and salivation (" response ").

In instrumental conditioning one observes fluctuations in some physiological activity and reinforces the tendencies in one direction with a reward and/or in the other direction with a punishment. For instance, one can condition heart beat, which varies in rate, by rewarding the subject with a pleasant noise, a picture or money, if his average rate during any period is higher than the mean, and by punishing him with a discord, etc., if it is lower than the mean (Schwartz *et al.*, 1971). With instrumental conditioning in human subjects it is often difficult to tell if the conditioning achieved is cognitive, or autonomic, or a mixture; but, regardless of this, the end result is an alteration in a normal physiological function.

With sweating we have seen that classical conditioning should be possible since there is an unconditioned stimulus (heat) and a response (sweating), but the delay time, the effects of humidity, etc., and the variability between subjects and within one subject from day to day, indicate that such conditioning would be time-consuming and expensive in terms of climate chambers and the number of subjects needed. We therefore attempted instrumental conditioning of palmar sweating, which is free from most of these limitations.

To avoid experimenter bias and to automate the system as far as possible, we designed an experiment in which the subject received an increased frequency in shocks when the amount of water loss from the palms increased, and in the control subject a decreased frequency in shocks when the amount of water loss from the palms increased; the results of these experiments were consistently negative. It seemed possible that the subjects could not distinguish between these different shock frequencies and that the conditioning was not successful because the subjects could never completely avoid punishment.

To test this we set up the second conditioning situation described in the *Experimental* section. In the subjects whom we attempted to condition downwards (i.e. lower sweat rate) we found that the mean sweat rate for 10 min decreased after the first trial, and in some cases no active sweating could be observed in any of the subsequent trial situations where shocks could be received. That is, these subjects managed to avoid any shocks within a small number of trials, generally within 3 or 4.

Thus we can say that, under these experimental conditions, the subjects " learned " to decrease their sweat rate markedly. Whether this " learning " was autonomic, cognitive, or only a side effect from some other learned activity that affects sweating (the muscle tone, heart rate, blood vessel constriction, etc.), cannot yet be determined. However, further investigations, which are in progress, should clarify these points. We can also say that, at least in some subjects, it is possible to reduce the spontaneous sweat rate on the palm of the hand by the technique that we have described. This reduction in sweating was very marked, and one of the subjects, who had not been told the purpose of the investigation, spontaneously offered the information that since he had taken part in the experiments his hyperhidrosis was greatly reduced. A more accurate experimental approach might be possible via the various frequencies apparent in the sweat pattern. This would require on-line autocorrelation of the sweat response, with subsequent Fourier transformation to produce a frequency spectrum which could then be conditioned. We are currently examining these possibilities.

We should like to thank Mr van Wamel and the staff of the electronics department of the Medical School of the University of Nijmegen for developing the shock apparatus; the illustration department for the pictures; Dr Hulstein of the Nijmegen University and Dr van Olst of the University of Amsterdam for helpful discussions.

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Comment

PROSTAGLANDINS AND THE EPIDERMIS

Prostaglandins are a group of 20-carbon carboxylic fatty acids incorporating a 5-member cyclopentane ring. Molecules designated prostaglandin E or prostaglandin F have, respectively, an oxygen atom or a hydroxyl group attached to the ring at the C9 site. This chemical difference confers dramatically differing pharmacological properties on the 2 groups of prostaglandins.

Although prostaglandins are widely distributed throughout the body, only extremely low concentrations are found in most tissues. One reason for this is that most tissues, as well as containing prostaglandin-synthesizing enzymes, also contain enzymes which degrade prostaglandins to inactive products. Thus, until the recent advent of refined techniques for extraction, chemical analysis and assay, little was known about the character and role of prostaglandins.

Skin contains both prostaglandins and prostaglandin synthetases. The ability of skin enzymes to catalyse the conversion of essential fatty acid substrate into prostaglandins was first demonstrated by Jessup *et al.* (1970), and later confirmed by Van Dorp (1971) and Greaves and McDonald-Gibson (1972). Van Dorp's data also showed that most of the prostaglandin synthetase activity was located in the epidermis. Both our own experiments (Greaves and McDonald-Gibson, unpublished results) and those of Van Dorp, indicate that the concentration of prostaglandins in normal skin is extremely low. Thus, any local increase in the concentrations of prostaglandins in skin in pathological or physiological conditions is presumably the result of increased local biosynthesis.

There is increasing evidence that prostaglandins influence the development and maturation of epidermis. Kischer (1967, 1969) has studied the effects of several different prostaglandins on cultures of chick embryo skin. He has shown that prostaglandins E_1 and B_1 stimulate epidermal cell proliferation and keratinization, but inhibit down feather organ development. These effects were specific since other prostaglandins as well as the essential fatty acid prostaglandin precursors, arachidonic acid and linoleic acid, did not reproduce the epidermal changes. Prostaglandin B_1 has additional interesting effects on skin cultures (Kischer and Keeter, 1970). If prostaglandin B_1 is incubated with cultures for 2 days or more, defects appear in the basement membrane which are later followed by full-thickness perforations of the epidermis. The relationship of these epidermal defects to the presumptive feather-sites is uncertain. If these results can be repeated with epidermis from other species, including man, they may have important implications in the pathogenesis of abnormalities of hair growth and of ulcerative skin conditions.

Approaching the same problem in a different way a number of workers have attempted to relate skin lesions in essential fatty acid-deficient laboratory animals to a reduction in prostaglandin concentrations in skin. Hansen *et al.* (1954) and Menton (1968) observed that dogs and mice fed on essential fatty acid-deficient diets developed thickening of the epidermis, hyperkeratosis, increase in epidermal mitotic activity, hair loss and increased sebaceous gland activity. These cutaneous changes preceded any other manifestations of essential fatty acid deficiency. The skin abnormalities were reversed by feeding the animals arachidonic acid and linoleic acid—the essential fatty acid precursors of prostaglandins. Administration of other fatty acids did not affect the lesions. Although attempts to reverse the skin lesions by administration of prostaglandins have not so far been successful, (Gottenbos *et al.*, 1967; Kupiecki *et al.*, 1968), this may be related to selection of the wrong prostaglandins, wrong dosage or wrong route of administration.*

Although work on the relationships of prostaglandin to epidermis is only in the earliest stages there are already enough data to suggest that prostaglandins may have an important role in the control of mitotic activity in epidermis and structures derived from it. Cellular concentrations of cyclic adenosine monophosphate (cyclic AMP) are sensitive to changes in local concentrations of prostaglandins (Butcher *et al.*, 1968) and there is evidence from *in vitro* experiments that epidermal cell proliferation is regulated by Cyclic AMP (Voorhees and Duell, 1971). It is thus reasonable to speculate that investigation of the relationship of essential fatty acids, prostaglandins and Cyclic AMP in proliferating epidermis may provide interesting information on the factors controlling replication of epidermal cells.

* ADDENDUM

Since the foregoing account was written, Blank has described experiments by Ziboh and Hsia in which scaly skin lesions in fatty acid-deficient rats have been cleared by topical application of prostaglandin E2.

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Review

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A REVIEW OF THE RECENT LITERATURE ON DISEASES OF THE VULVA—PART III

CARCINOGENESIS AND TUMOURS

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CARCINOGENESIS

THE wart virus and HVH may be concerned in carcinogenesis. Hay and Cole (1969, 1970) point out the importance of other chronic infections in inducing squamous carcinoma in Jamaica. In 52 patients with carcinoma, 34 had had LGV, granuloma inguinale or both, and they were younger than the group where there was no known previous infection. The authors conclude that the former infection probably, and the latter possibly, predisposes to malignancy, and thereby confirm the findings of Saltzstein *et al.* (1956) in a much quoted paper.

The administration of stilboestrol seems to predispose to vaginal adenocarcinoma (*Lancet*, 1971). Feingold and Koss (1969) report a middle-aged woman with chronic myeloid leukaemia who was given Busulfan for several years. She had an abnormal cervical smear and a vulval carcinoma *in situ* with superficial invasion, and at her death was found to have cytological abnormalities in various tissues and adenomata of the pancreas and kidney.

Lemke (1967) invokes light as a carcinogenetic factor in cows. From the findings of Brodey and Roszel (1967) in dogs, it is clear that the pattern of malignancy may vary from species to species; Cherry and Glucksmann (1968) and Glucksmann and Cherry (1970a, b), however, have carried out a sophisticated and extended study of carcinogenesis in the cervix, vagina and vulva of rats which, even if not directly applicable to humans, must influence future work. The rats were subjected to various endocrine influences, by hormone additives or by castration. Neoplasms (sarcomata, squamous and basal cell carcinomata) were induced by paintings of dimethylbenzanthracene, the number of paintings necessary being used as a measure of the threshold of the tissues to malignant change. Some conclusions are that the threshold has a particular level for various tumours, that administration of thyroactive substances can accelerate the formation of squamous carcinomata of the vulva up to a certain level of carcinogenic stimulus but that, with increasing stimulation, the incidence falls off, and that the threshold for carcinogenesis is lower in castrate than in intact animals.

TUMOURS

Sarcoma

All forms are rare. Thiers *et al.* (1969) describe a fibrosarcoma of Darier and Ferrand (i.e. dermatofibrosarcoma protuberans) at the vulva.

Witt (1967) reports 3 sarcomata of the vulva—a rhabdosarcoma, a leiomyosarcoma and a reticulosarcoma. Recorded cases (only 150) are discussed and 12 more presented by Disala *et al.* (1971). Histological classification is difficult, but they considered their 12 cases to comprise 5 leiomyosarcomata (one perianal), 3 rhabdosarcomata, 3 neurofibrosarcomata (one perianal) and one fibrosarcoma.

In infancy a vulval sarcoma is usually of the botyroid (grape-like) type, an embryonic form of rhabdosarcoma, with masses forming in the vagina and bladder and presenting as a tumour at the vulva. The lesions are highly malignant and involve many pelvic organs. One such case with a blue-red vulval mass, in a child aged 9 months, is described by Moonen and Janknegt (1970); after pelvic exenteration, the child had then survived for 11 years. James *et al.* (1969) had a similar case, histologically proven, presenting as a smooth swelling of one labium in a child aged 6 months. The authors believe that only one other case, with the lesion originating in this way at the vulva in infancy, has been previously reported.

Malignant Melanoma

Ostergard and Townsend (1968) reported a malignant melanoma of the urethra resulting in a tumour at the orifice and satellites on the labium minus. This they treated successfully (follow-up one year) by initial cryosurgery and then orthodox radical surgery; at operation, the malignant cells were ghost-like and necrotic. Two cases of malignant melanoma of the vagina are reported by Laufe and Bernstein (1971). Malignant melanoma of the vulva is discussed by Yackel *et al.* (1970) in a survey of the literature and 29 of their own cases. Malignant melanomata are said to comprise about 2% of malignant vulval tumours. Survival was related to glandular involvement, but not to size of the tumour. Two were amelanotic. The lesions tended to be near the midline, whereas squamous carcinomata tend to be more laterally placed.

Basal Cell Carcinoma

Bean and Becker (1968) reported one case and reviewed the literature. About 90 examples had then been reported. They are said to form only 2–3% of all vulval malignancy. The usual site is the labia majora, but the lesion may occur on the labia minora and clitoris. Their patient, aged 56, had no history of having taken arsenic.

Palladino *et al.* (1969) added 4 cases and reviewed, using more stringent criteria of diagnosis and follow-up, 61 previous cases. They noted a recurrence rate of more than 20% and advocated wide excision. Thirteen cases were associated with primary malignancy elsewhere. No mention was made of arsenic, but 2 of the patients had skin malignancy elsewhere and one a squamous carcinoma of the vulva. No evidence of glandular metastases was found, and the authors concluded that in no adequately documented and treated case had death seemed to be consequent upon the basal cell carcinoma.

Paget's Disease

Although rare (Caputo and Califano (1970) say about 100 cases had been published up till then), Paget's disease of the vulva stimulates a large amount of work, largely from the point of view of its disputed pathogenesis. A summary of the conflicting views is given by Gardner and Kaufman (1969); it may represent an intraepithelial metastasis from an underlying sweat gland tumour, a tumour of epidermal cell rests with glandular potential, or arise multicentrically in epidermis and/or in underlying sweat glands.

Koss *et al.* (1968) reported 10 cases, seen over 34 years; 8 were followed up for periods of 3–21 years. None was diagnosed clinically, and all occurred in middle life. In 6 patients where the material was adequate, a careful search revealed an underlying carcinoma of the sweat glands in 5. The prognosis seemed to be that of this carcinoma, with the extent of epidermal changes of less importance. Of the 10 patients, 4 had a carcinoma elsewhere—breast, cervix, vagina, and a basal cell carcinoma of the lip. They concluded that the Paget's cells, with a tendency to form glandular acini and with characteristic histochemical properties, arose from the sweat glands rather than in the epidermis itself.

Koss and Brockunier (1969) use electron microscopy to investigate the origin of the cells, and conclude that the carcinoma, when found, stems from eccrine rather than apocrine glands. Their grounds for the conclusion are the morphology of the cells and in particular the fact that secretory and non-secretory cells can be distinguished, which latter feature they believe is characteristic of cells of eccrine origin. Moreover, although in their earlier paper the site of any carcinoma found was cautiously described as in sudariparous glands, they here say it could be seen to be in eccrine glands. They also claim that the Paget cells are not identical ultramicroscopically with those shown in Paget's disease of the breast by the same method. Caputo and Califano (1970) have also carried out an electron microscopical study of Paget's disease of the vulva, and they on the contrary concluded that the 2 types of cells found to comprise Paget's cells are identical to those identified in Paget's disease of the nipple. They quote earlier studies to the same effect, not, however, mentioning the work of Koss and Brockunier (1969).

Vogel and Ayers (1970) report 2 cases where no underlying carcinoma could be found even after careful search, and Lecart *et al.* (1970) a further similar case. The latter authors wrongly allege that Koss *et al.* (1968) ascribed the lesion to an underlying apocrine gland carcinoma.

Boehm and Morris (1971) report 5 more cases, 3 without and 2 with an underlying tumour, said to be apocrine in origin. One of their own had Paget's disease of the breast, and they cite a similar example in the literature. From previous cases and their own, they conclude that, when there is a carcinoma, especially when there are glandular metastases, the prognosis is poor. As regards the aetiology, they favour an apocrine gland origin, which may be pre-malignant and not necessarily malignant from the beginning. Kawatsü and Miki (1971) describe axillary and genital Paget's disease in a male; one objection to the theory of apocrine gland origin has been that axillary lesions are rare.

Greenwood and Minkowitz (1971) report a patient who had an intraductal carcinoma of the breast, with histological changes of Paget's disease, and later developed cutaneous secondary deposits. One deposit on the arm was excised and its histological appearance, including histochemistry, suggested Paget's disease. It therefore seemed likely to have resulted from direct invasion, particularly as apocrine glands would not be found at this site, so at least one way in which the condition could appear is by infiltration from an underlying carcinoma rather than by an *in situ* change of epidermal cells. The authors suggest Paget's disease may be of multiple aetiology.

In a patient with Paget's disease of the vulva, Mullick *et al.* (1970) examined cultures of vulval fibroblasts, fibroblasts from elsewhere (arm) and blood cells. An atypical X chromosome has been noted in the leucocytes of patients with malignant or pre-malignant disease of the uterus and breast, and similar changes were found here. Changes seen in the chromosomes of fibroblasts from the lesion were akin to those seen in fibroblast chromosomes of a distant site (the arm) when the latter were challenged by a known oncogenic agent; it was not possible to say whether the changes were basically genetic or environmental. Sachs (1970) found significant similarities in the DNA of Paget's lesions of nipple and vulva.

Bowen's Disease

Bowen's disease at the vulva, although sometimes used as a general term for an intraepithelial carcinoma, has been reported as a distinctive clinical and histological entity in a woman with multicentric and pigmented lesions by Wilson (1971), who mentions a similar case in the male reported by Lloyd (1970). In neither case was there a history of arsenic ingestion.

Squamous (Epidermoid) Carcinoma

Kobayasi (1969) has made a study by electron microscopy of the changes in the dermo-epidermal junction in an invasive squamous cell carcinoma of the vulva. He describes how protrusions from basal cells extend through the junction, how carcinoma cells are to be seen in the middle layer of the epidermis, and how malignant cells seem to be stretched along elastic fibres in the dermis.

Lymphography has been used with good effect to detect metastases by Comas *et al.* (1969). Malkasian *et al.* (1968) and Haffner and Frick (1970) describe the failure, complete or almost so, of chemotherapy.

Large series of cases with discussion of treatment and prognostic factors are described by Barclay and Collins (1967); Goplerud and Keettel (1968); Collins *et al.* (1970b); Rutledge *et al.* (1970); Singleton *et al.* (1970); and Franklin and Rutledge (1971). Rutledge and Sinclair (1968), Collins *et al.* (1970a) and Marchac *et al.* (1971) deal particularly with intraepithelial lesions. All authors stress that radical surgery offers the best results, that delay between onset and diagnosis is often unduly long, and that the incidence of other primary carcinomata, especially in the same region, is notable.

It is difficult, however, to assess the significance of a second primary in what is often a middle-aged or elderly patient, and to exclude a recurrence. Ostergard and Morton (1967) are impressed by their 24 cases of multifocal genital cancer and review previous examples. Arneson and Schellhas (1970), however, find that the frequency of carcinomata elsewhere in patients treated for carcinoma of the cervix is not more than that in a control population. Woodruff *et al.* (1969) took as a subject a patient with carcinoma *in situ* changes of the cervix, vulva and perianal area. The chromosomal picture differed in these lesions, suggesting an independent origin for the neoplasms. Jimerson and Merrill (1970) provide the most helpful analysis of the literature. They believe that multicentric squamous malignancy involving the cervix and vulva is significant, and apply stringent criteria to exclude metastases. They survey the literature and add 15 cases. There seems no doubt that the association is significant. It is in this field of multicentric multiple malignancies, where the tumours arise in the same tissue and organ or in a paired organ (as opposed to simple double primary tumours, which may often be the result of chance), that further study is likely to be rewarding. It seems unlikely that irradiation for the first-occurring tumour is of aetiological importance.

MISCELLANEOUS TUMOURS

A lymphoid hamartoma of the vulva, perhaps the first at this site, is reported by Kernen and Morgan (1970) in a woman aged 20; the tumour is usually found in the mediastinum and thorax. Histologically it showed lymphoid cells and corpuscle-like aggregates of epitheloid cells. Martini (1968) described a vulval tumour which he regarded as a lymphangioendothelioma. Fisher and Orkin (1970) report bilateral plaques of lymphangiomatous vesicles in the vulva and genito-crural folds, developing in an elderly woman some years after pelvic irradiation. There was no obvious lymphoedema, so they postulate a direct effect of irradiation on the tissue, and contrast this case with those reported of lymphangiectatic changes following surgery and subsequent lymphoedema. However, the patient had had recurrent attacks of fever, and tender plaques in the area, and it seems likely that the lymphangiectasia was related to some blockage of lymphatics by the irradiation.

Multiple angiokeratomata of the vulva, rarely reported but perhaps not so rarely occurring, are discussed by Blair (1970). They are probably analogous to scrotal angiokeratomata.

Carneiro *et al.* (1971) report 3 women with multiple vulval syringomata, confirmed histologically. One patient is that reported, with a photograph, by Gardner and Kaufman (1969). No other cases could be found; a previous report was of pubic rather than vulval lesions. Two patients had no lesions elsewhere; none had a family history of the condition. These lesions too, the authors suggest, may be more common than would appear from the paucity of reports. Zalla and Perry (1971) have reported an analogous case in a man with multiple syringomata on the penis and none elsewhere.

Granular cell myoblastomata on the contrary seem to be reported fairly often at the vulva. Two recent reports, citing earlier ones, are of Ponnampalam (1968) and Radman and Bhagavan (1969); the latter authors mention a case in the literature where a vulval lesion became malignant.

An enormous vulval cyst, containing sebaceous material and weighing 6000 g, was reported by Sachdeva and Narang (1968). Hart (1970) reports 3 cases of paramesonephric mucinous cysts, all in the labia minora, which is the usual site for this relatively rare vulval lesion. They occur more often in the vagina. They are thought to arise from displaced Mullerian (glandular) epithelium, and a raised progesterone level may be important in their enlargement.

According to Gardner and Kaufman (1969) endometriosis at the vulva is indeed rare, only 36 cases in the perineum and 16 of the vulva itself having been reported up to 1964. Most of the lesions were secondary to local surgery, usually uterine curettage; that more deposits are not found following child-birth may depend on hormonal factors at that time. In reporting an adenocarcinoma of the vagina, Bamford (1967) suggests it may have arisen in an area of vaginal endometriosis. The subject crops up only once in the more recent literature, when Duperrat and Ficheux (1970) report 6 cases affecting the vulva, only one of which seemed likely to have been primary.

Woodworth *et al.* (1971) discuss the papillary hidradenoma of the vulva, based on clinical and histological findings in 69 cases collected over 60 years at the Mayo Clinic. The authors doubt previous reports of malignancy in these tumours on the grounds of unconvincing or absent histological details. (Such a report, although not cited by these authors, is that of Householder (1969), whose patient was a Negro.) Although they are commonly believed to arise from apocrine glands, it is possible that they are derived from eccrine glands or undifferentiated cells. Their rarity in the Negro, where apocrine glands are thought to be more numerous, and at other sites where apocrine glands are found, is anomalous. However, histologically they resemble intraduct papillomata of the breast. While the usual incidence after puberty is held to support the theory of apocrine origin, it should be noted that Dalla Pria and Domenichini (1968) have reported (in a paper not cited by Woodworth *et al.*, 1971) 5 cases of vulval hidradenomata, including one in a child aged 4 years. The problem in fact is somewhat similar to that of the provenance of Paget's disease. Two apocrine cystadenomata, which may be analogous tumours, are reported (for the first time at this site) on the prepuce by Ahmed and Jones (1969).

Reticuloses

Reticulum-cell sarcoma at the vulva has been reported by Schiller and Madge (1970), who review past reports of involvement of the genital tract in general by malignant lymphomata.

Eosinophilic granuloma presenting as a vulval tumour was reported by Borglin *et al.* (1966) with a review of previously reported cases, then totalling 17. A further case, with electron microscopy, was described by Toth *et al.* (1969); and another by Craig and Castleman (1970), again with electron microscopy which showed tennis racquet-like structures, said to be typical of the condition, in the cytoplasm of the histiocytes.

Bartholin's Gland

Kelly (1969) describes the practical treatment of an abscess of the gland; as does Goldberg (1970) who recommends a special self-retaining catheter for its drainage.

Smith Foushee *et al.* (1968) review benign lesions, and report 5 cases, perhaps hamartomatous; 2 had bilateral adenomata, 2 a unilateral adenoma and cyst; one had thrombosed vessels, which may have been part of a haemangioma.

Carcinoma of the gland is well recognized. According to Dodson *et al.* (1970), carcinomata may be missed if abscesses of the gland are marsupialized without a check being made on the histology, particularly in the middle-aged patient. They report 4 cases. The tumours are very malignant. From cases already in the literature, it seems that epidermoid (squamous) carcinoma and adenocarcinoma comprise roughly half each, with a few rarities occurring from time to time. There may be a separate group categorized as adenoid cystic carcinoma. Van Nagell *et al.* (1969) have described a case—which they think may be the first recorded—of an adenoacanthoma, occurring in part of a carcinoma which was squamous in type but had also areas of adenocarcinoma.

Breast Tissue

Ectopic breast tissue may occur at the vulva, unilaterally or, more rarely, bilaterally. The tissue may vary with menstruation. Levin and Diener (1968) report a case of bilateral lesions, enlarging in pregnancy and hence excised. Hassim (1969) surveys the previous literature, citing Smith Foushee and Pruitt (1967), who added 2 cases to the previously reported 21 of ectopic breast tissue; of this total, 8 had fibroadenomata, 6 lactating tissue, 4 adenocarcinomata, 4 cystic disease, and one a sarcoma. He reports an African woman who had tender masses in the labia majora; histologically, each showed fibroadenomatous changes in breast tissue. Chaphekar and Wagh (1967) report a swelling histologically suggesting breast tissue, unilateral in a woman aged 40.

A more bizarre example of ectopic breast tissue was reported by Brightmore (1971)—a cyst in an ectopic breast, mainly represented by a nipple and an areola, in the posterior axillary line of a man. The author discusses the view that such a lesion may be an atavistic remnant of the milk line or, alternatively, a development in a modified sweat gland. Such considerations may have relevance to the discussions on Paget's disease.

Pseudo Tumours

Capraro *et al.* (1970) point out that a prolapse of the urethral mucosa in childhood may present as a vulval mass. El-Hammady and Ghoneim (1971) report a somewhat similar appearance secondary to complete inversion of the bladder. Anderson (1968) had a patient aged 64 with a pudendal hernia resulting in a labial swelling. Cleary *et al.* (1968) report a patient with a labial swelling associated with ascites and a patent canal of Nuck.

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Society Proceedings

ROYAL SOCIETY OF MEDICINE

SECTION OF DERMATOLOGY

President: Dr H. R. VICKERS

Thursday, November 18th, 1971

Oral-Facial-Digital Syndrome.—Dr R. J. CAIRNS.

A girl aged 4½ years.

History.—She was born with a cleft palate, and hair which was stiff and rubbed off easily. Within a few weeks multiple facial milia appeared. When she was first seen at the age of 5 months the following features were noted: lank hair and areas of baldness; naevus flammeus on forehead, Unna's naevus over occiput; scattered facial milia affecting particularly the cheeks and forehead; cleft palate, bifid tongue, multi-tufted buccal frenulae, tumour of tongue; clinodactyly. In 1969 a plastic repair of the cleft palate was performed (Mr McLaughlin). At the same time the tumour of the tongue was removed and the tongue-tie relieved. Further surgery is envisaged to complete the repair of the palate. The child is somewhat retarded and has difficulty with speech, for which she is receiving therapy. Hearing tests are normal. During the last year the facial milia have resolved spontaneously, leaving pitted scars. Excessive hair fall continues. The naevus on the forehead has cleared and that on the occipit is resolving.

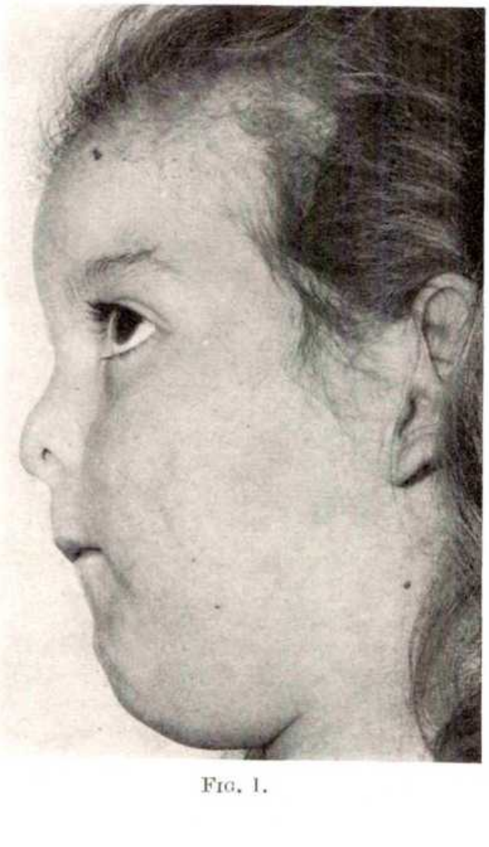

FIG. 1.

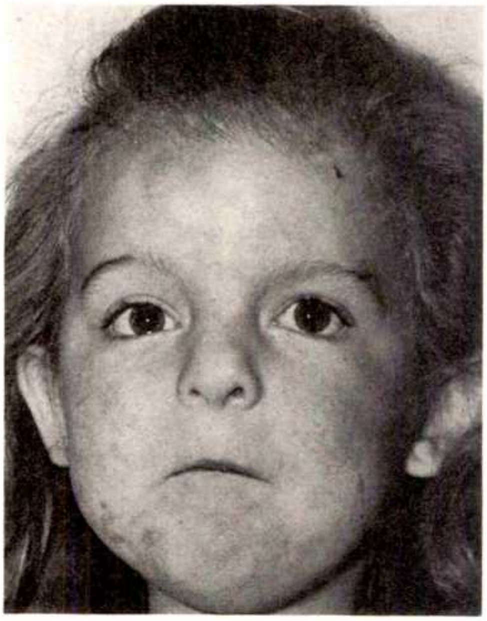

FIG. 2.

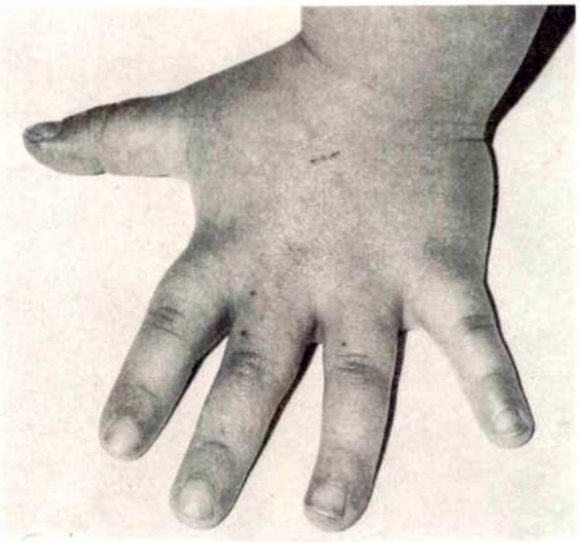

FIG. 3.

Family history.—She is the fourth of 5 children. No history of similar disorder in siblings or relatives. No consanguinity or twins.

On examination.—The profile is characteristic with a hooked pug nose and a short philtrum (Fig. 1). The upper lip is narrow (Fig. 2). Dentition is defective. The scalp hair is lank. All the fingers are short; the fourth left finger shows clinodactyly (Fig. 3). The right second and third toes are webbed and the left second and third toes show clinodactyly.

Investigations.—Normal: buccal smear, urinary amino-acids, full blood count, serum cholesterol. Abnormal: skull x-ray—irregular ossification with thinning in right parietal region. Histology of tumour of tongue (Dr J. Burn): a nodule in the tongue consists of mucous salivary glands in a connective tissue stroma which includes bundles of plain muscle and fibres of striated muscle. A heterotopic mucous salivary gland.

Comment.—This rare syndrome was first described by Papillon-Léage and Psaume (1954). Since then over 125 cases have been reported. It is X-linked and lethal in the male. The tumour of the tongue in this case closely resembles that reported by Doege *et al.* (1964). The milial cysts probably represent maldevelopment of the pilosebaceous follicles, (Solomon *et al.*, 1970).

Lichen Myxoedematosus.—Dr J. J. H. GILKES for Dr R. S. WELLS.

A woman aged 63.

History.—Two years ago the patient's skin became increasingly pruritic and infiltrated, especially on the neck, antecubital fossae and lower abdomen, giving it a coarse texture. Her voice became croaky and she developed dysphagia 9 months ago; in addition there was weakness of the proximal muscle groups and congestive cardiac failure.

Family history.—Her father is Italian and she has always been relatively hyperpigmented. The pigmentation has increased where the skin has become infiltrated.

On examination.—Her skin was strikingly infiltrated and pigmented, especially on the sides of her neck (Fig. 1 and 2). The only area of normal skin was on her back. The thyroid gland was not palpable and there was no lymphadenopathy. On admission she was in gross congestive cardiac failure which responded to treatment, but her liver remained enlarged.

There was marked weakness of the proximal muscles of the limbs but no abnormality in the central nervous system.

Investigations.—Full blood count, E.S.R., liver, thyroid and adrenal function tests normal. No auto-antibodies detected. Serum protein electrophoresis showed a thickened band in the gamma globulin range. IgA and IgM within the normal range, but the IgG was raised at 2200 mg %. Immunoelectrophoresis showed the increased IgG to be IgG lambda. Bone marrow revealed a 12% increase in plasma cells but was otherwise essentially normal. Barium swallow showed a dilated oesophagus with no peristalsis of the middle and lower thirds. There was evidence of an old anteroseptal infarct on the E.C.G. while the E.M.G. indicated an acute and widespread myositis.

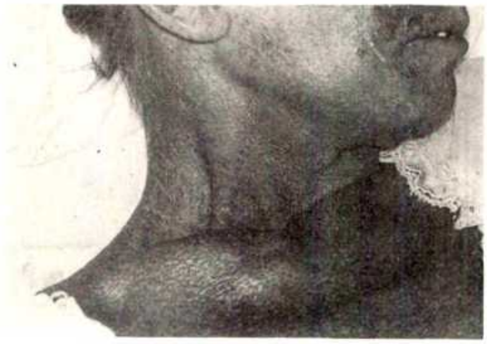

FIG. 1.

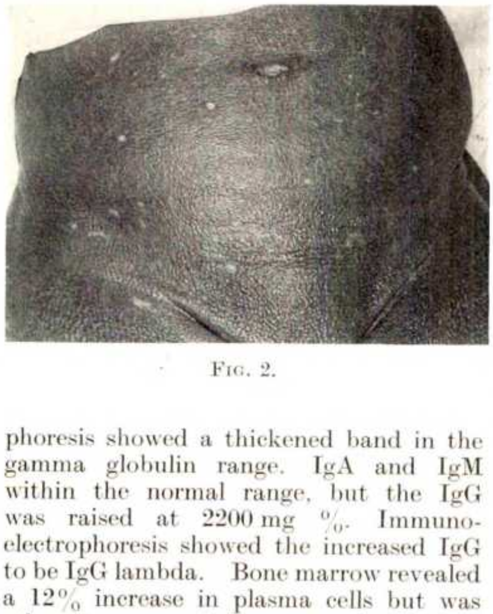

FIG. 2.

Skin biopsy from her neck showed a number of foci in the upper dermis in which fibroblasts, sometimes showing a slightly whorled appearance, were abnormally numerous. The section stained well with Alcian blue, only in low molarities of magnesium chloride, indicating that the mucopolysaccharide was only weakly acid and probably one of the carboxylated mucopolysaccharides. Mucopolysaccharides extracted from the skin biopsy showed an abnormal band on electrophoresis which was hyaluronidase labile. Muscle biopsy showed many fibres which were abnormal in that they had a a central nucleus and showed peripheral vacuolation and infiltration by a few neutrophils and macrophages. The serum creatine phosphokinase was raised at 80 u/l but there was no urinary myoglobin. Urine—concentrated 50 times had no Bence-Jones protein. The uronic/creatinine ratio suggested a mucopolysaccharide excretion rate of at least 3 times normal for this age and electrophoresis showed the major increase to be in the heparan sulphate fraction. Another unusual feature was the occurrence of a small fraction which corresponded with hyaluronic acid and was hyaluronidase labile. Hyaluronic acid is not normally found in the urine after early childhood. (These estimations were carried out by Dr G. Manley, Exeter University).

Comment.—Lichen myxoedematosus may remain confined to the skin. However, several reports indicate that some of these patients have had systemic complications. Rudner *et al.* (1966) described 2 patients with lichen myxoedematosus who died of myocardial infarction. A female patient who died following a myocardial infarction discussed by McCarthy *et al.* (1964) showed an extremely basic globulin in both the serum and the skin. Basic monoclonal IgG with light chains of the type lambda (λ) have been shown in the sera of several patients with lichen myxoedematosus. Rowell *et al.* (1969) described multiple protein abnormalities using disc electrophoresis, in addition to the previously recorded abnormal basic globulin fraction. There were no abnormal plasma cells in the bone marrow of these patients but one patient reported by Perry *et al.* (1960) had multiple myeloma. Lichen myxoedematosus has been reported to be associated with thyroiditis and dermatomyositis (Von Nagy *et al.*, 1962), but there are no other reports of myositis occurring in a patient with lichen myxoedematosus. Donald *et al.* (1953) described a patient with lichen myxoedematosus who had progressive dysphagia.

Summary.—A patient with typical cutaneous lichen myxoedematosus who has an abnormal globulin in her serum and also multiple systemic complications involving her heart, oesophagus and skeletal muscle.

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The following cases will be published in *Proc. R. Soc. Med.*

Gross Hyperkeratosis of the Palms and Soles, Pemphigoid and Epilepsy.—Dr R. A. J. EADY (for Dr E. J. MOYNAHAN and Dr J. F. WILKINSON).

Acrodermatitis Enteropathica with Lactose Intolerance.—Dr P. J. MILLA (for Dr E. J. MOYNAHAN).

Waardenburg's Syndrome in Two Siblings, Both Their Parents and Their Maternal Grandmother.—Dr T. J. DAVID and Dr R. P. WARIN.

PORPHYRIA CUTANEA TARDA

Proceedings of the International Symposium, Rome 1970.

A one day Symposium on Porphyria Cutanea Tarda (PCT) was held in Rome on October 24th, 1970 at the S. Gallicano Dermatological Institute. It was organized by Professor Paolo Nazzaro, the Director of the Institute who has also edited the Proceedings which have recently appeared in a special issue of the *Bollettino dell'Istituto Dermatologico S. Gallicano* (1971, **7**, No. 1.)

Porphyria Cutanea Tarda of the symptomatic variety associated with chronic alcoholism and liver damage is relatively common in Italy, particularly in the region round Rome and in the north, near Brescia. Clinical contributions to the Symposium were from several major centres in Italy as well as from the Universities of Paris and Barcelona. The principal topics discussed were the clinical symptomatology and therapy of PCT, the histopathology of the dermatological lesions, and the problem of hereditary disposition which appears to be present in some of these patients. The emphasis throughout the meeting was on the clinical aspects of PCT.

After a general introductory paper by Nazzaro, several rare clinical forms of the disease were described by Puissant, Piñol-Aguadé, Mascaro and their co-workers: these included a scleroderma-like syndrome, certain unusual cutaneous lesions and various ocular manifestations. The histopathology of the cutaneous lesions commonly observed in PCT in the areas of skin exposed to sunlight was discussed by Tosti who distinguished, on the basis of studies conducted both at the optical and at the scanning electron microscopes, 2 main kinds of pathological changes: the elastosis of the middle dermis, an essentially irreversible lesion, and the protein-rich oedema in the upper dermis, which may disappear with the improvement of the disease.

The dominant role of the liver in the pathogenesis of the disease was discussed by Ventura and collaborators, who pointed out the constant involvement of the liver function in these patients and the frequent deposition of iron within the liver, the significance of which is still not clear. The structural changes observed with the electron microscope in the liver of patients with PCT were discussed by Picardi: the earliest detectable changes were in the mitochondria in the form of paracrystalline inclusions, followed by the appearance of siderotic pigment within the liver cells and, later, of autophagic vacuoles in the more seriously damaged hepatocytes.

The beneficial effect of blood letting in PCT was confirmed by several authors. Levi and Alessi compared the effect of this treatment with that of administering hydroxychloroquine for several months at a weekly dose of 400 mg: both treatments were found to be safe and effective. A transient increase in the serum transaminase levels was observed at the beginning of treatment with hydroxychloroquine, but this was followed by a gradual and steady improvement of both the cutaneous symptoms and the tests of liver function. A decrease in the urinary excretion of porphyrins was noted after 3–4 months of treatment.

Mazza, Battistini and Prato discussed the results of an extensive study on the faecal excretion of porphyrins in 8 patients with PCT (belonging to 8 different families) and in 119 of their blood relations. In the case of all but one patient, a significant increase of coproporphyrin and/or of protoporphyrin was observed in the stools of several asymptomatic blood relations. The distribution of the biochemical abnormality within the families suggests that it is transmitted as a dominant, non-sex linked, Mendelian character. These observations, which are in agreement with previous reports by the same authors and by others, point to the existence in at least some of the patients with PCT of an underlying genetic disposition to develop the disease. Additional factors like liver damage and increased deposition of iron in the liver appear to be necessary however to determine the onset of the clinical symptoms.

The problem of the pathogenesis of PCT appears therefore to be a complex one. The real outstanding points which await elucidation are the nature of the genetic disposition and the way in which it interacts with liver damage and disordered iron metabolism to produce the disease as well as the exact biochemical lesion which is responsible for increased formation of porphyrins in the liver.

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Correspondence

The Physical Properties of Skin in Cutis Laxa

SIR,—Since Dr Black and Professor Shuster (1971) have used your columns to voice criticisms of our paper, perhaps we may be permitted to reply to their letter point by point.

1. They do less than justice to McConkey *et al.* (1963) who showed that fat was barely detectable in a skin-fold raised over the fourth metacarpal shaft on the dorsum of the hand.
2. For technical reasons it is necessary to perform the *in vivo* skin elasticity test on the dorsum of the fore-arm where, unfortunately, it is not possible to raise a fat-free fold; for this reason it is not possible to estimate the skin thickness directly at the site tested. Nevertheless a study of cadaveric skin comparing the thickness of the skin of 13 paired specimens taken from both the dorsum of the fore-arm and the dorsum of the hand overlying the fourth metacarpal bone showed no significant difference (Grahame, 1970).
3. Though we maintain that the Harpenden caliper is the best *clinical* method available for estimating skin thickness we acknowledge that Black's (1969) radiological method may be more accurate. There are, however, certain practical objections, since in the study in question several patients were visited in their homes in remote parts of the country.
4. On the question of tethering in relation to spherical displacement we have presented elsewhere (Grahame, 1970) convincing evidence that significant skin tethering does not occur, and that in the situation in question a circle of skin behaves for practical reasons as a free-moving diaphragm, thus disproving Vlasblom's hypothesis that it is not possible to measure skin elasticity *in vivo* by means of a suction cup device.

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The Glands of Montgomery

SIR,—I have read with interest the article on The Glands of Montgomery by William Montagna and Jeung Soon Yun (*Br. J. Derm.* (1972) **86**, 126). It would perhaps have made the article even more informative if some reference had been made to Montgomery and his pioneer work. William Fetherston Montgomery (1797–1859) was King's Professor of Midwifery in the University of Dublin.

Breast changes as an aid to diagnosis in pregnancy were first adequately described in English by Montgomery in a volume published in London, in 1837 entitled *An Exposition of the Signs and Symptoms of Pregnancy* (Paternoster Row, London: Sherwood Gilbert and Piper). Describing breast changes he stated, " I cannot say positively what may be the earliest period at which this change can be observed, but I have recognised it fully at the end of the second month at which time the alteration in colour is by no means the circumstances most observable but the puffy turgescence (though as yet slight) not alone of the nipples, but of the whole of the surrounding disk, and the development of the little glandular follicles are the objects to which we should principally direct our attention ".

The major point of Montagna and Yun's report is that the glands of Montgomery are integral parts of the mammary apparatus. They also say that they often become functional during lactation and milk can be expressed from them. Montgomery stated, " These follicles or tubercles of the areola, although by many considered merely as sebaceous glands have really a much more important character, and more intimate connexion with the peculiar structure and function of the breasts, and hence might naturally be expected to display an active sympathy in any condition of the system which called into action the peculiar structure and function of these organs, which is the secretion of milk for the support of the new being for which purpose certain previous changes in the glands and ducts are necessary. Now it appears that these areolar tubercles are intimately connected with the lactiferous tubes some of which can be traced into them and opening on their summit so that in pregnant women a sero lactescent fluid may be often distinctly perceived issuing from them and in nurses they have been observed to pour forth drops of perfect milk ".

This information is taken from an article by Professor J. B. Fleming on " Montgomery and the Follicles of the Areola as a sign of Pregnancy ", *Irish Journal of Medical Science*, 6th series, No. 485, 169–182. May 1966.

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Jejunal Biopsies in Bullous Pemphigoid

Sir,—The only previously reported study on histological changes in the small intestine in patients with bullous pemphigoid is that of Marks and Shuster (1969) in which selection of patients for study was based on clinical and histological considerations. In view of the difficulty which can at times arise in the differentiation between bullous pemphigoid and dermatitis herpetiformis on these grounds, we have carried out a study on 7 patients in whom the clinical diagnosis of bullous pemphigoid has been confirmed by direct and indirect immunofluorescent techniques (Beutner and Jordan, 1964; Jordan *et al.*, 1967; Beutner *et al.*, 1968; Cormane and Szabo, 1970). Direct examination showed in every case immunofluorescence of the basal lamina characteristic of bullous pemphigoid, the dilutions of sera at which fluorescence was observed by the indirect technique are shown in Table I. In no case was there immunofluorescence with IgA, as previously reported in dermatitis herpetiformis (Chorzelski *et al.*, 1971).

Table I

Sex and age	Skin biopsy	Positive Ab titre in dilution of	Jejunal biopsy
M 72	BP or DH	1/1280	Normal
M 80	BP	1/640	Few broad leaves
F 65	BP	1/200	Normal
F 49	BP or DH	1/160	Normal
M 72	DH	1/640	Few broad leaves
F 80	BP or DH	1/80	Normal
F 49	DH	1/80	Normal

Biopsy of the small intestine was performed using the Crosby capsule; this was passed by mouth and after radiological confirmation of correct positioning, a sample of jejunal mucosa was taken from the proximal jejunal area. In every case normal mucosal architecture was observed, confirming the previous report of lack of jejunal villous atrophy in patients with bullous pemphigoid.

An interesting feature of this group of patients is the fact that in only 2 cases was histological examination of an early bullous lesion considered diagnostic of bullous pemphigoid, of the remaining 5 lesions 2 were considered to be more in keeping with dermatitis herpetiformis, and 3 were not diagnostic of either condition.

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Books

Cutaneous Lesions of the Lower Extremities. M. H. SAMITZ and A. S. DANA (1971) Philadelphia: Lippincott. Pp. 198 + xii, 135 illustrations in colour. Price £14.00.

After an introduction of about 25 pages on basic principles of dermatology, the authors discuss in 11 chapters most of the disorders seen on the lower limbs. Stress is almost entirely on the clinical aspects of the diseases, although there are certainly some references to the pathology. At the end of each chapter is an excellent list of references.

If one accepts the fact that it is justifiable, for example, to write a book on only part of an organ, such as the ascending colon or the rectum, it is justifiable to produce a book like this. As the authors indicate, it will be of particular benefit to students of chiropody (*syn.* podiatry) for whom it is eminently suited. The appeal to medical students, postgraduate students and general practitioners, who are also mentioned in the preface, may not be quite so compelling. The text is reliable and well-written. The numerous colour illustrations are not only excellent but helpful, although they must add to the not inconsiderable cost. No book is perfect, and it may be constructive, if another edition is contemplated, to suggest to the authors several improvements. It is illogical to urge that size " should be expressed in millimetres and centimetres " but to refer to plantar warts as " varying in size from a pinhead to a half-dollar " and at a later stage to refer to metric and imperial measurements in the same sentence. Text books in English are widely read throughout the world, and it seems a pity to use terms such as " grade-school children " and to refer to proprietary names of drugs which may be unintelligible outside the country of origin of the book. Apart from these minor criticisms and doubt regarding the wisdom of including longish case reports in a book of this nature, this well-produced volume can be recommended.

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Regulation of Mast Cell Formation. Ed. G. CSABA (1972). (Studia Biologica Hungarica, vol. II.) Budapest: Akademiai Kiado. pp. 147, 76 illus. Price £2.00.

There is now a vast literature on the structure, biochemistry, pharmacology and pathology of mast cells, but evidence on the origin and development of these cells is limited and conflicting. Professor Csaba's monograph is valuable for two reasons: firstly he defines some of the more important problems related to genesis of mast cells and secondly he draws attention to potentially useful experimental approaches—particularly into relationships between biogenic amines and transformation of precursor cells into definitive mast cells.

Most of Csaba's book is taken up by summaries of his numerous experiments in this field. It might have been better if he had concentrated on more detailed evidence from fewer experiments since his monograph frequently gives the impression that he draws unjustifiably bold conclusions from mainly indirect evidence. His evidence that mast cell genesis occurs mainly in the thymus and lymph nodes is based principally on evidence from the effects of corticosteroids on mast cell counts in these tissues, using basic dyes for identification. One is left with the nagging doubt that apparently increased counts could alternatively have been due to steroid-induced changes in permeability of mast cells to dye. In any case as Csaba himself points out later in the book, metachromatic dyes do not distinguish between mast cells and other cells which have ingested metachromatic material. A similar criticism applies to his experiments on S^{35} incorporation into thymic tissue following corticosteroid treatment.

Csaba's evidence suggests that the presence of serotonin is an essential prerequisite for transformation of a precursor cell into a mast cell, but it is worrying that the rat mast cell model he uses is almost unique in its possession of significant amounts of serotonin. He advocates the interesting idea that corticosteroid induction of mast cell genesis depends on induction of serotonin biosynthesis through combination of steroid with histone " repressor substance ". The consequent " depression " of genes controlling production of enzymes responsible for serotinin synthesis results in increased cellular serotonin formation. His evidence for this appears to be based on autoradiographic studies of localization of corticosteroid in mast cells and histochemical demonstration of histone in mast cell granules. Csaba ambitiously concludes that this evidence " confirms the theory of induction mechanism ".

Csaba is right to emphasize the probable endocrine function of mast cells although he does not mention the newer evidence on stimulus-secretion coupling mechanisms in mast cells which strongly supports his view. Although he describes the appearance of increased " mast cells " in the blood of corticosteroid-treated rats he gives virtually no discussion of the relationships between tissue mast cells and blood basophils—one of the most controversial topics related to genesis of mast cells.

Despite these criticisms I found the book extremely stimulating. The electron micrograph and histological illustrations are frequent and of excellent quality and include several colour plates. There are also useful subject and author indexes and ample references. The book is very reasonably priced and should be bought by all interested in the morphology, function and clinical significance of mast cells.

M. W. GREAVES

Quarterly Review

THERAPEUTICS XIX

Lipid Lowering Agents

Xanthomatous skin lesions usually indicate abnormal lipid metabolism. However, exceptions to this are 50% of patients with xanthelasma, patients with histiocytic or reticulo-endothelial disorders and diffuse plane xanthomata.

It is now possible to classify the majority of lipid disorders on the basis of alterations in the proteins concerned with their transport (Fredrickson *et al.*, 1967). Biochemically six types of hyperlipoproteinaemia can be identified.

Type I is characterized by an increase in chylomicra due to an insufficiency of lipoprotein lipase. Type II is further subdivided into a and b. In both there is an increase in the beta-lipoproteins and a raised serum cholesterol, but in type IIb there is also an increased pre-betalipoprotein and raised triglyceride level. The underlying defect in primary type II disease is not established. Secondary causes of type II disease include hypothyroidism, obstructive biliary disease and the nephrotic syndrome. An abnormal protein which on electrophoresis appears between the beta and pre-beta bands is characteristic of type III, and both cholesterol and triglyceride levels are raised. In type IV, pre-betalipoproteins are increased and there is elevation of the triglyceride level. Type V appears to be a combination of types I and IV with an increase in chylomicra and pre-betalipoproteins. Currently, the diagnosis is best determined by fasting serum cholesterol and triglyceride levels with electrophoresis of the lipoproteins. Classification helps in the selection of specific therapy.

Xanthomata may be present in any of these disorders. With the exception of plane xanthomata on the palmar surface of the hands which appear to be limited to the type III disorder, the xanthomata are not diagnostic. Eruptive xanthomata occur most frequently in types I, III and V, whilst tuberous lesions appear mainly in types II and III. Xanthelasma may be associated with type II but in 50% of cases no demonstrable abnormality of lipoproteins is present (Slack and Borrie, 1971). Only a relatively small number of patients with hyperlipoproteinaemia develop xanthomata. Xanthomata developing in childhood are usually due to a homozygous type II or the rare type I disorder.

It is now accepted that a high serum cholesterol level is associated with an increased incidence of premature atherosclerosis and early death from myocardial ischaemia. The incidence of premature atherosclerosis in all the hyperlipoproteinaemias has not yet been fully established. Type II is certainly associated with a very high incidence of early coronary artery disease (Slack, 1969). In types III and IV there is an increased incidence in both coronary and peripheral vascular disease but the mortality rate is not as high as in type II (Fredrickson *et al.*, 1967; Slack, 1969) (Table I).

TABLE I.—*Types of Hyperlipoprotinaemia* (*after Fredrickson* et al., *1967*)

	Fasting serum				
Type	Cholesterol	Triglyceride	Electrophoresis	Main clinical features	Treatment
I	N to +	+++	Chylomicra +++. Other lipoproteins decreased	Rare. Abdominal pain and colic. Eruptive xanthomata, hepatosplenomegaly	Low fat diet
IIa	+++	N	β lipoprotein ++ to +++.	Very common. Often secondary to hypothyroidism, hepatic disease, nephrotic syndrome. Accelerated severe atheroma, mainly coronary. Xanthelasma and tuberous tendinous xanthomata	Diet. Cholestyramine. Nicotinic acid. Clofibrate less effective
IIb	+++	+	β lipoprotein ++ to +++. Pre β lipoprotein +		
III	++	+ to ++	Broad β band	Rare. Peripheral atheroma common. Mild diabetes. Xanthomata as in II and IV + palmar creases	Diet. Clofibrate
IV	+	++	Pre β lipoprotein ++ to +++	Common. Often obese and diabetic. Increased incidence of atheroma—not as marked as in II and III. Xanthomata rare. Hyperuricaemia. Abdominal pain ± pancreatitis	Low carbohydrate diet. Clofibrate. Anti-diabetic therapy.
V	+	++	Chylomicra + to ++. Pre β lipoproteins ++	Common. Very similar to IV	Diet. Clofibrate.

N—normal, +—raised, ++—very raised, +++—extremely raised.

Therapy is directed at lowering the raised lipid levels, reducing the incidence of premature atherosclerosis, relieving other symptoms such as abdominal pain in patients with type I, IV or V disorders and resolving xanthomata.

Theoretically, the raised serum lipid levels might be reduced by lowering the intake of lipid, decreasing hepatic synthesis, promoting excretion or any combination of these three methods.

Dietary control is the most important part of management of any patient with a raised lipid level. The type of dietary restriction is related to the lipid abnormality present. Thus in the rare type I, severe restriction of fat intake is the mainstay of treatment. In type II restriction of cholesterol intake will substantially reduce the betalipoprotein level (Fredrickson *et al.*, 1967). Reduction of total calorie intake and in particular carbohydrate content will reduce lipid levels in types III, IV and V.

In many cases dietary management is insufficient to reduce the raised lipid levels to normal and the addition of lipid lowering drugs in indicated. Levy *et al.* (1969) stresses that the combination of drugs and diet is an additive effect.

Clofibrate.—(ethyl-α-14-chlorophenoxy-isobutyrate) was found to reduce total lipid and cholesterol concentrations in blood and liver in rats by Thorp and Waring (1962). The complexity of its mode of action was reviewed by Thorp (1966), who reported that clofibrate occupies linkage points on albumin normally available for the transport of fatty acids. Feinberg *et al.* (1967) found that clofibrate inhibits the incorporation of triglycerides into lipoproteins. Triglyceride metabolism may also be altered by interference with the rate of hepatic secretion (Duncan *et al.*, 1964) or by an increased oxidation or removal rate (Spitz, 1965; Ryan and Schwartz, 1965). Clofibrate also reduces the rate of cholesterol synthesis in the liver (Avoy *et al.*, 1965; Nestel *et al.*, 1965).

Clinical studies have shown clofibrate to be particularly effective in lowering raised triglyceride levels and to a lesser extent cholesterol levels. It is therefore the drug of choice, in addition to dietary control, in patients with type III hyperlipoproteinaemia, and is effective to a lesser extent in type IV and V. Strisower *et al.* (1968) observed a mean reduction of 70% in pre-betalipoproteins in patients with type III disorder on clofibrate. Borrie (1969) confirmed this finding. The xanthomata in type III also appear to respond well to this drug. In Borrie's study (1969) both eruptive and palmar xanthomata disappeared completely in all 18 cases treated, and there was a 50% reduction in the size of tuberous xanthomata. Levy *et al.* (1969) and Brehm (1968) also report complete regression of xanthomata in type III patients on clofibrate.

In patients with type II disorder clofibrate produces a less marked fall in the raised cholesterol level (Jepson *et al.*, 1969; Segall *et al.*, 1970) and the regression of xanthomata is more variable.

Whether these biochemical changes induced by clofibrate influence the occurrence of ischaemic heart disease or peripheral vascular changes remains to be established. Zelis *et al.* (1968) showed some increase in peripheral blood flow in type III patients treated with clofibrate. Of great interest have been the results of recent trials of clofibrate in ischaemic heart disease (Physicians of the Newcastle-upon-Tyne Region, 1971; Research Committee of the Scottish Society of Physicians, 1971). An overall decrease in myocardial infarction was seen and a reduction in sudden death. Whilst the serum cholesterol and triglyceride were reduced the protection from myocardial ischaemia appeared to be unrelated to change in the serum lipid levels. It is possible that prevention of myocardial ischaemia is effected, at least in part by the action of clofibrate on plasma fibrinogen, platelet adhesiveness or its action on the vessel wall (Editorial, 1972).

Clofibrate produces few side effects, minor gastrointestinal upsets, weight gain and transient elevation of transaminase levels (Bender, 1970; Mason and Perry, 1965; de Gennes *et al.*, 1965). El-Mehairy *et al.* (1970) found no evidence of liver damage. It may potentiate the effect of oral anticoagulants and interfere with coagulation (Oliver, 1964). Langer and Levy (1968) observed a curious syndrome of myalgia and malaise associated with a raised creatine phosphokinase and transaminase levels in some patients on clofibrate therapy.

Nicotinic Acid.—Like clofibrate, this drug reduces plasma cholesterol and triglyceride levels (Altschul *et al.*, 1955; Parson and Flinn, 1957). It inhibits free fatty acid mobilization from adipose tissue and may reduce hepatic synthesis of the triglyceride rich prebetalipoproteins (Carlson and Orö, 1962). Nicotinic acid may also inhibit cholesterol synthesis (Merril, 1958; Hardy *et al.*, 1960) and increase excretion of neutral steroids of endogenous origin (Miettinen, 1968).

To be effective as a lipid lowering agent nicotinic acid has to be given in as large a dose as 4–6 g daily. The corresponding alcohol, beta piridylcarbinol is converted in the liver to nicotinic acid (Raaflaub, 1966) and has the same metabolic effects as nicotinic acid (Schön *et al.*, 1963; Zöllner and Gudenzi, 1966) but, is active in a smaller dose of 0·3–1·2 g daily.

The main use of nicotinic acid and its derivatives is in the treatment of type II patients with hypercholesterolaemia. Regular administration results in a fall in serum cholesterol level of 20–25% (Berge *et al.*, 1961; Parson, 1961; Fitzgerald *et al.*, 1964). The maximum effects are obtained within 1–2 months of treatment but may not always be sustained. Cholesterol levels return to pre-treatment levels within 4–6 weeks of stopping treatment (Zöllner and Gudenzi, 1966). Xanthomata may regress (Carlson, 1969; Zollner *et al.*, 1971). However, Heiberg (1969) observed regression in only 4 of 17 patients treated. Zeller *et al.* (1966) reported a decrease in liver size and a return of liver function tests to normal in 20 patients with fatty infiltration of the liver and hyperlipidaemia treated with nicotinic acid.

The usefulness of nicotinic acid is limited by the high incidence of side effects. The most troublesome and frequent being a cutaneous flush due to peripheral vasodilatation. This makes it unacceptable to many patients. Other side effects include gastrointestinal upsets (Berge *et al.*, 1961; Fitzgerald *et al.*, 1964; Zöllner and Gudenzi, 1966). Liver function tests may be disturbed and jaundice has been reported (Berge *et al.*, 1961). Nicotinic acid may decrease glucose tolerance after prolonged administration (Berge *et al.*, 1961; Zöllner *et al.*, 1971).

Cholestyramine.—This drug has an entirely different mode of action to the two previous drugs. Chemically it is a large polymer containing quarternary ammonium groups attached to a styrene divinylbenzene skeleton. In the bowel it binds bile salts and promotes their enterohepatic circulation (Hashim and van Itallie, 1965). Binding of bile salts reduces their effective concentration and hence the absorption of exogenous lipid. Loss of bile salts results in the greater use of cholesterol from body stores to replace them and hence a fall in serum cholesterol level. However, this effect may only be temporary since endogenous cholesterol synthesis may accelerate to keep pace with this reduction.

Cholestyramine is most effective in type II patients with hypercholesterolaemia. The serum cholesterol level is reduced by 10–30% (Fallon and Woods, 1968; Gherondache and Pincus, 1964; Casdorph, 1967). Fredrickson (1971) observed a fall of between 50–60% in cholesterol levels in patients on low cholesterol diet and cholestyramine. Xanthomata may regress (Hashim and van Itallie, 1965; Howard *et al.*, 1966; Mishkel, 1967). Whether this reduction in serum cholesterol level will be associated with less ischaemic heart disease is not yet established.

Cholestyramine is beneficial in the treatment of pruritus of primary biliary cirrhosis and cholestatic jaundice (Datta and Sherlock, 1966).

Side effects are frequent and mainly gastrointestinal (Holmes, 1970; Gherondache and Pincus, 1964). High doses may cause steatorrhoea and reduce the absorption of fat soluble vitamins (Holmes, 1970). Questran appear to be a more palatable and acceptable form of cholestyramine.

Other drugs.—Oestrogens and D-thyroxine are also known to be effective in reducing serum cholesterol levels. The high incidence of side effects generally contraindicates their use.

The metabolism of fat is poorly understood. However, certain lipoprotein abnormalities are associated with raised serum lipid levels and a high incidence of atheroma. Dietary restriction and lipid lowering agents will undoubtedly reduce these raised levels, and in some instances, cause regression of xanthomata. Whether this fall in lipid level is also associated with a reduced incidence of atheroma is not firmly established. Furthermore, as these drugs are not without side effects and the therapy for a lifetime, the justification of their use awaits further evaluation.

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News and Notices

Royal College of Physicians of London

The following members of the British Association of Dermatology were recently elected to the Fellowship of the Royal College of Physicians of London: S. R. Wood, J. D. Everall, N. A. Thorne, R. R. M. Harman, W. H. Jopling, C. M. Ridley, J. M. Marks, R. S. Wells, G. A. Hodgson.

Postgraduate Medical School, University of Cambridge

The annual introductory Course on The Biology of Skin will be held in Churchill College from December 3rd to 9th, 1972. Applications should be sent to J. W. Woodcock, M.A., Secretary, Cambridge University Medical School, Hills Road, Cambridge.

Scottish Dermatological Society

The 145th Meeting of the Society was held in Stobhill General Hospital on Thursday, March 9th, 1972. The following papers were read and discussed:

(1) " Videotape Recording of Patients with Skin Tumours at a Combined Clinic ".
Dr J. C. P. Logan

(2) " The Influence of Bases on Skin Penetration by Steroids, using an *in vitro* Method ".
Dr R. S. Chapman

(3) " Current Concepts on the Role of the Langerhans Cell ".
Dr J. Hunter

XXIX Brazilian Dermatological Congress

This congress will be held at Niterói from October 16th–21st, 1972 under the presidency of Professor R. D. Azulay. Information may be obtained from the General Secretary, Dr R. Garrido Neves, Avenida Estácio de Sá, 123, Niterói, Rio de Janeiro, Brazil.

Acknowledgment

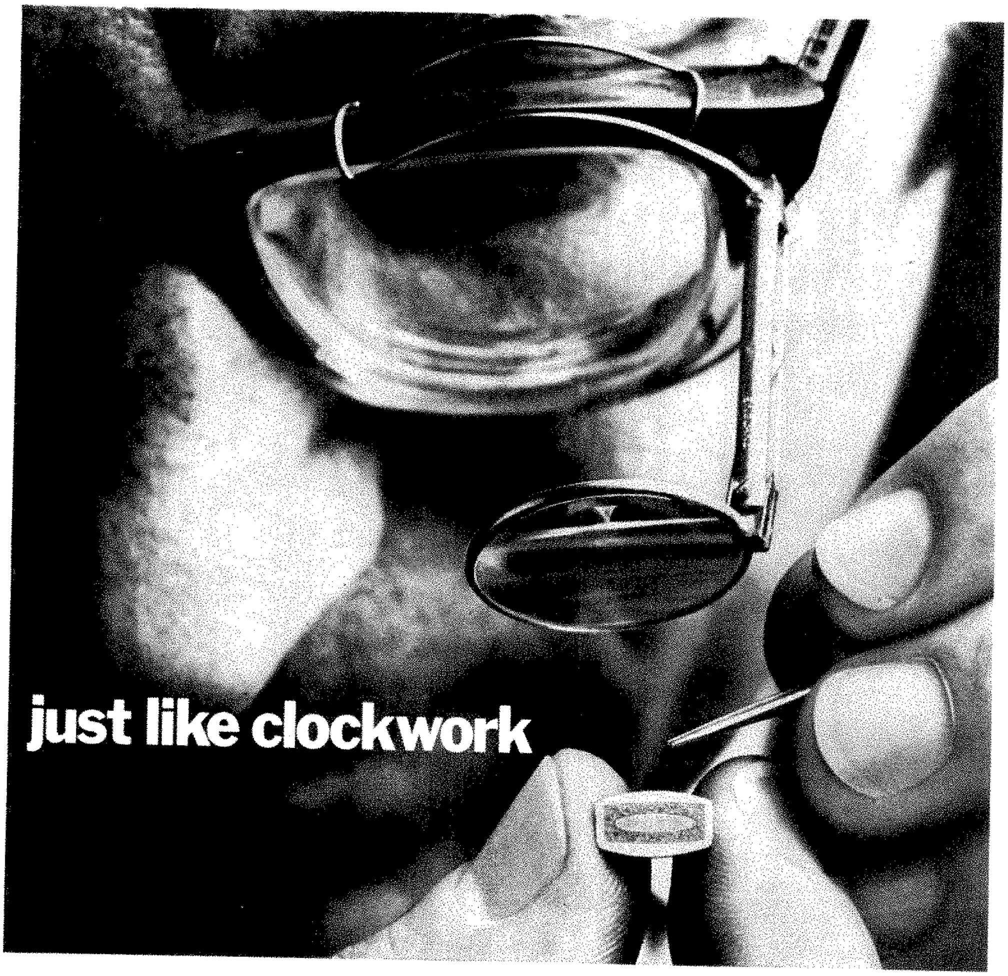
Dr T. W. Stewart and Miss D. Savage are grateful to Syntex Pharmaceuticals Ltd for covering the cost of the illustrations in colour in their recent article.

Appointments in Britain

Croydon Group of Hospitals

Dr Jeffrey Joseph Cream has been appointed Consultant Dermatologist to the Croydon Group of Hospitals.

Dr Cream qualified B.Sc., with Honours in Physiology from Manchester in 1958 and then proceeded to obtain his M.B., Ch.B. in 1961 and became M.R.C.P. in 1965. He was Registrar in Medicine in Cardiff for two years and at St Thomas' Hospital for a further year. He then became Registrar in the Department of Dermatology at St Thomas' Hospital in 1966, and in 1969 he was appointed Senior Registrar in the Department of Immunology in St John's Hospital for Diseases of the Skin. Finally he became Senior Registrar in Dermatology at the Middlesex Hospital, London in 1971. He has published numerous articles dealing mainly with Immunology, Cryglobulins and Vasculitis.



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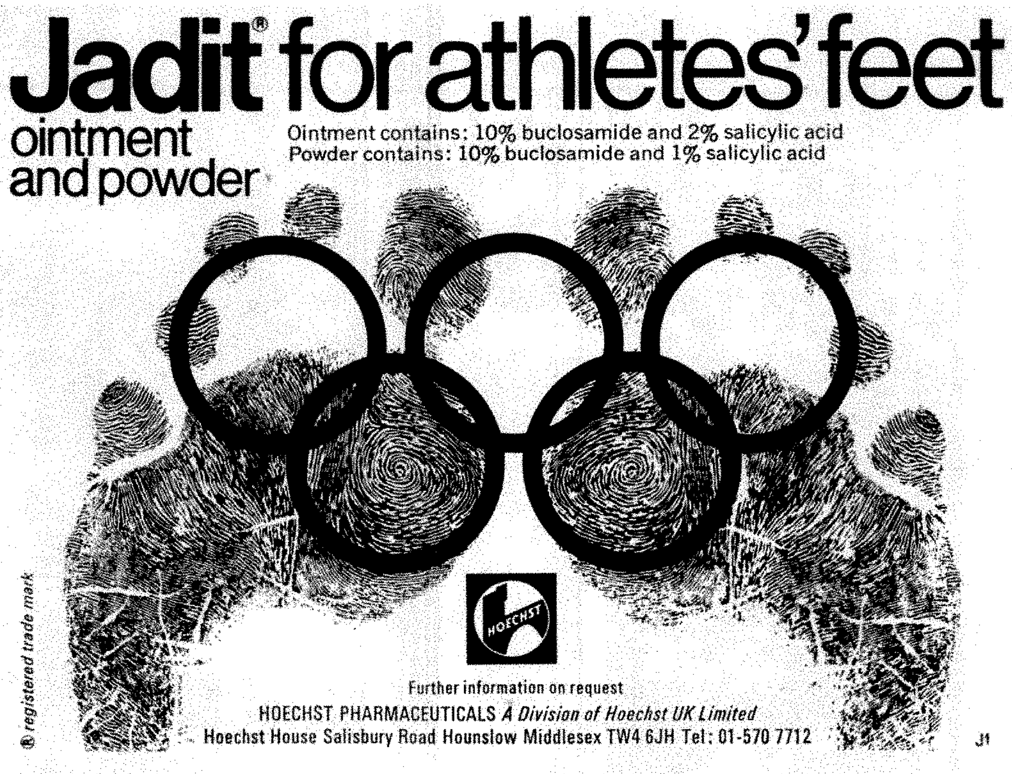
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CONTENTS (*Continued*)

Correspondence

The Physical Properties of Skin in Cutis Laxa
RODNEY GRAHAME and PETER BEIGHTON 176
The Glands of Montgomery
F. O. C. MEENAN 176
Jejunal Biopsies in Bullous Pemphigoid
N. K. SAIKIA, A. MCQUEEN and R. MACKIE 177

Books

Cutaneous Lesions of the Lower Extremities (Samitz and Dana); Regulation of Mast Cell Formation (Csaba (Edit.)) 179

Quarterly Review

Therapeutics XIX. Lipid Lowering Agents
E. FRENK, ANNE E. WALKER and I. R. EDWARDS 180

News and Notices 185

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

Cutaneous Polyarteritis Nodosa
Peter Borrie . . . 87
Cytology of Mycosis Fungoides and the Sézary Syndrome. Correlation between Light and Electron Microscopy
J. Gomez Orbaneja, E. Sanchez Yus, L. Diaz-Flores and P. Simon Huarte . . . 96
The Epidermal Component of Pityriasis Lichenoides
R. Marks and M. M. Black . . . 106
Toxoplasmosis and Pityriasis Lichenoides
N. B. Zlatkov and V. C. Andreev . . . 114
The Ultrastructure of Lichen Amyloidosus with Special Reference to the Epidermal Changes
Martin M. Black and Christopher J. Heather . . . 117
Familial Lichen Amyloidosis. Report of 19 Cases in 4 Generations of a Chinese Family in Malaysia
K. Rajagopalan and C. H. Tay . . . 123
A Quantitative Study of Erythrasma Lesions
Dorothy A. Somerville . . . 130
Earliest Features of the Strawberry Mark in the Newborn
Akira Hidano and Shinobu Nakajima . . . 138
Prognosis in Industrial Dermatitis
D. Burrows . . . 145
The Effect of Chronic Sun Damage on the Activity of Metabolic Enzymes of the Epidermis of Human Skin
D. Cerimele, S. Yamasawa and F. Serri . . . 149
Conditioning of Sweating. A Preliminary Report
B. R. M. Kuypers and D. W. K. Cotton . . . 154

Comment

Prostaglandins and the Epidermis
Malcolm W. Greaves . . . 161

Review

A Review of the Recent Literature on Diseases of the Vulva—Part III. Carcinogenesis and Tumours
C. M. Ridley . . . 163

Society Proceedings

Royal Society of Medicine, November 18th, 1971 . . . 171
International Symposium, Rome 1970 . . . 174

(*Continued on* p. iii *of cover*)

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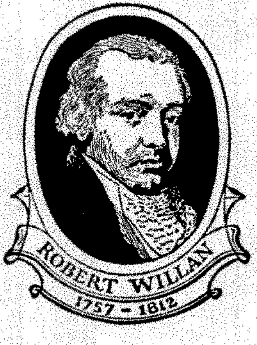

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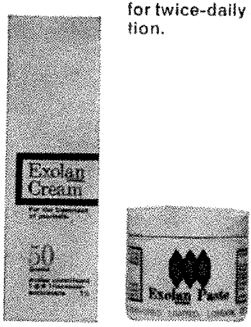

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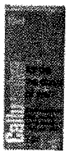

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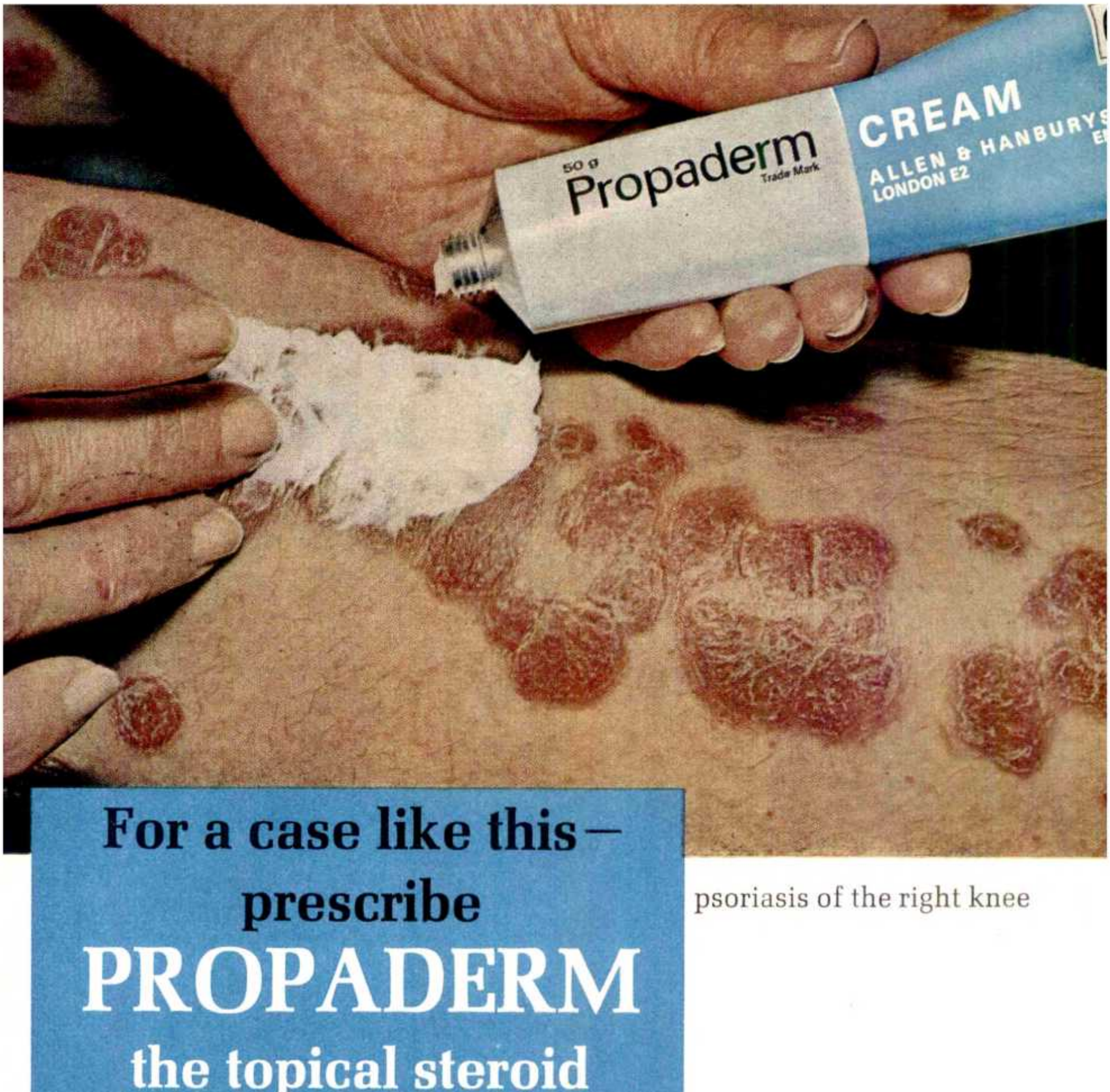

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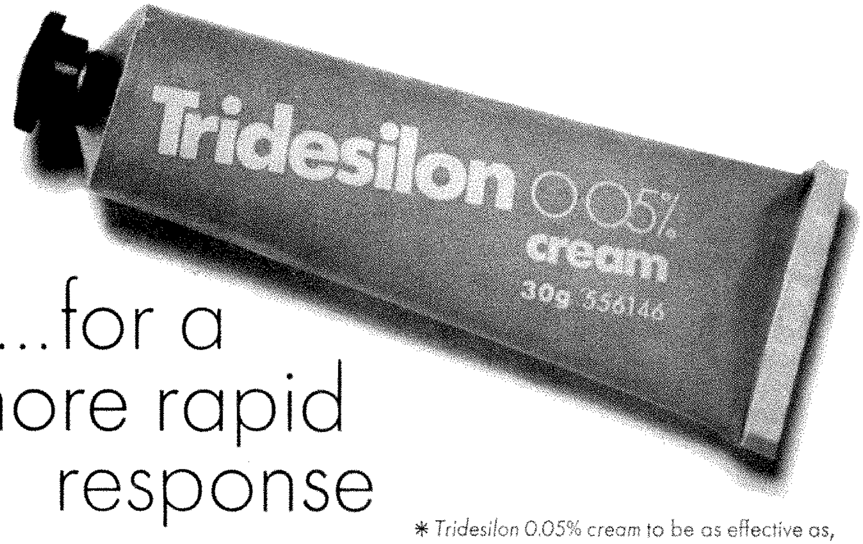

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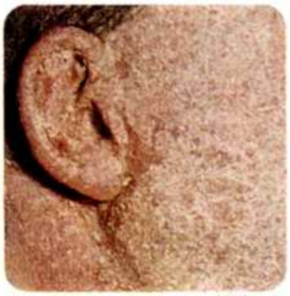
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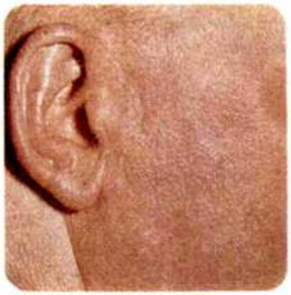
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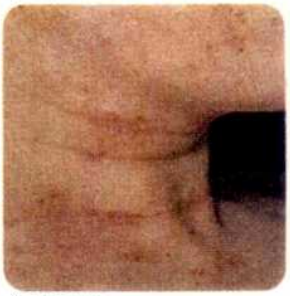
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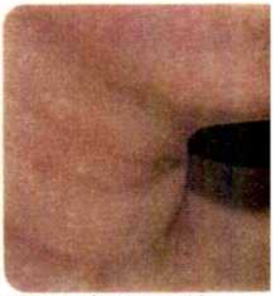
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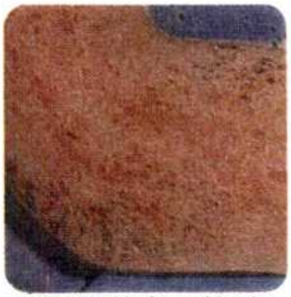
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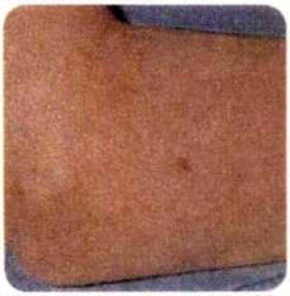
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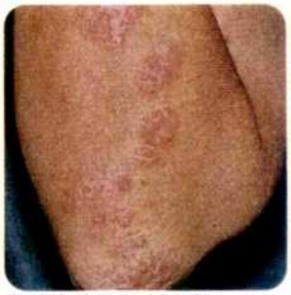
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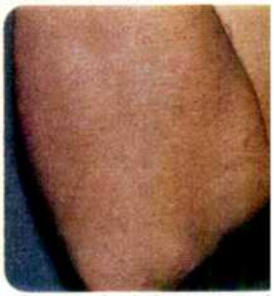
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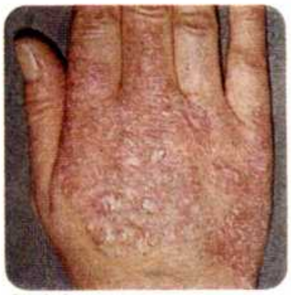
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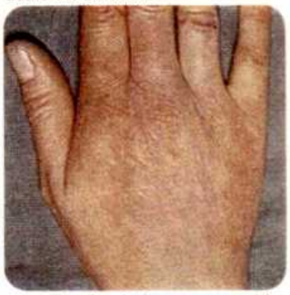
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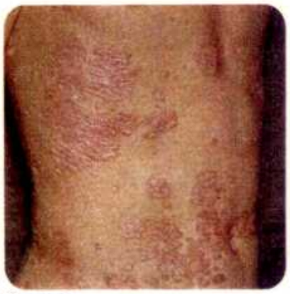
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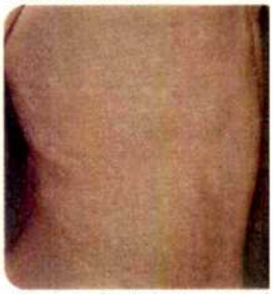
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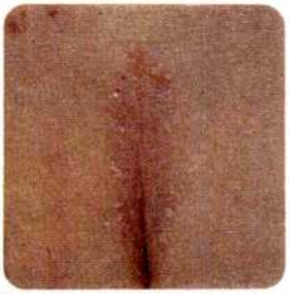
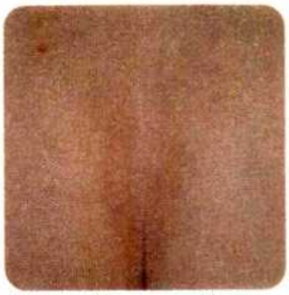

Flexural psoriasis of 15 years' duration


After 4 weeks' treatment with
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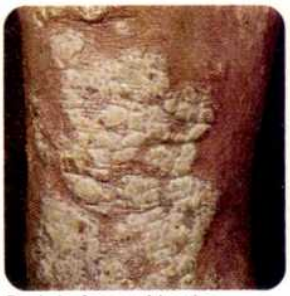
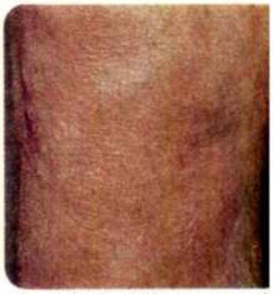

Psoriasis of 51 years' duration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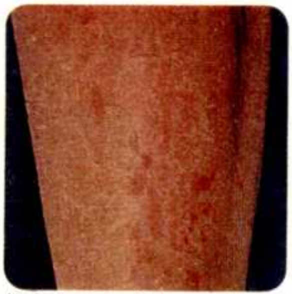
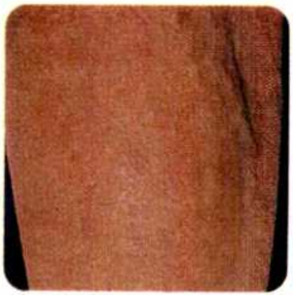
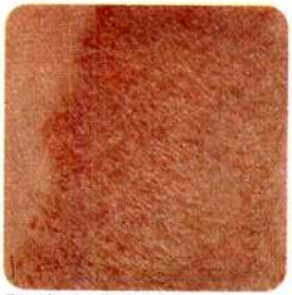
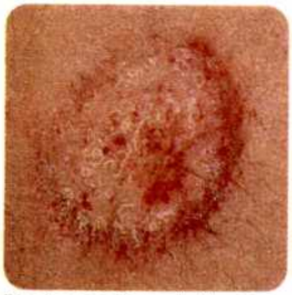

Varicose eczema of 4/5 months' duration


After 7 days' treatment with Betnovate Cream


Constitutional eczema

After 4 weeks' treatment with Betnovate Cream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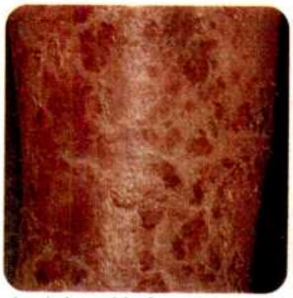
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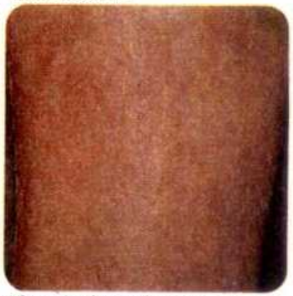
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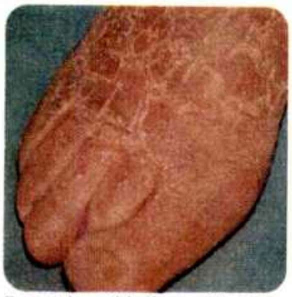
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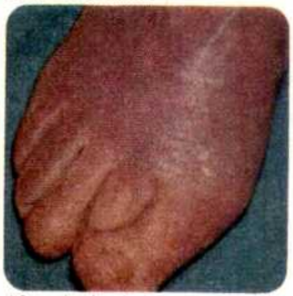
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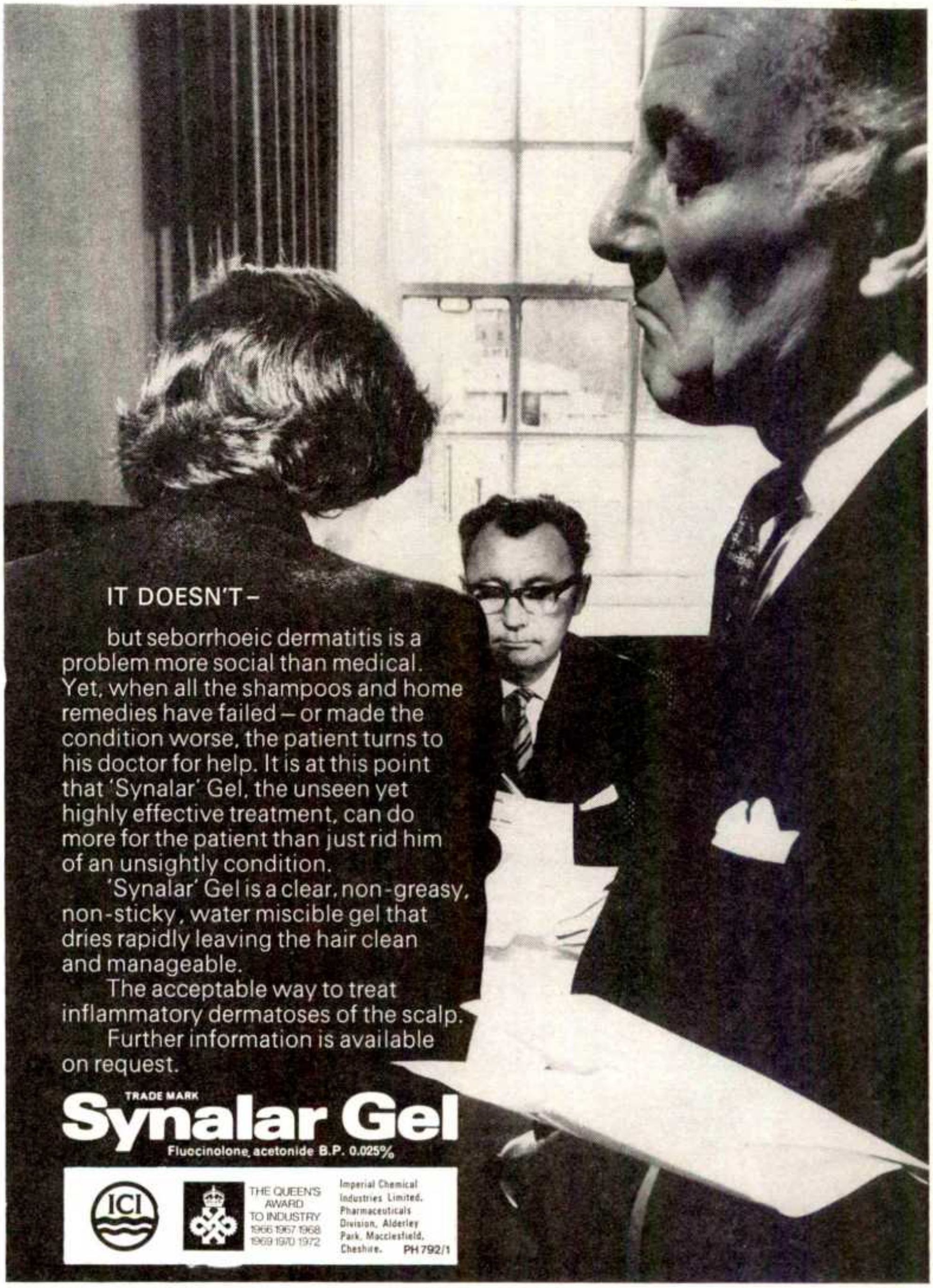

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Mitosis Research Laboratory, Birkbeck College, University of London,
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THE CONTROL OF EPIDERMAL THICKNESS*

WILLIAM S. BULLOUGH

Summary.—(1) Epidermal mass (thickness) is controlled by a complex mechanism in which the epidermal chalone plays a central role. The chalone inhibits epidermal mitotic activity by reducing the proportion of the basal cells that are in the mitotic cycle and the speed at which this cycle is completed. It also acts to reduce the speed at which the distal post-mitotic cells age and die.

(2) The response of the epidermal mass (thickness) to a rising mitotic rate (falling chalone concentration) is divisible into 3 separate phases.

(3) In Phase 1 (e.g. normal mouse epidermis) the mitotic rate is low. Mitosis occurs in the basal layer in such a way that the 2 daughter cells remain basal; the pressure so generated is relieved by the extrusion of a neighbouring basal post-mitotic cell. Any change in the mitotic rate is matched by an equivalent change in the rate of ageing of the extruded cells; thus no change in epidermal thickness occurs.

(4) In Phase 2 (e.g. normal human epidermis, hyperplastic mouse epidermis) the mitotic rate has increased beyond the point at which all the basal cells have entered the mitotic cycle; there are therefore no post-mitotic basal cells available to be extruded. From this point onwards the greater the mitotic rate (the lower the chalone content), the higher the lateral pressure within the basal layer, the greater the folding (or doubling) of this layer, the higher the numbers of basal mitotic cells per unit area of skin, the higher in consequence the number of distal post-mitotic cells (which are also ageing more quickly) per unit area of skin, and the thicker the epidermis. Thus epidermal mass (thickness) is a function of the degree of folding (or doubling) of the basal layer and of the increased rate of post-mitotic ageing. Towards the top of Phase 2 (e.g. psoriasis, papillomata) the chalone content of the whole epidermis has fallen to a low level, but the rate of cell gain still equals the rate of cell loss.

(5) In Phase 3 (e.g. epidermal carcinomata) the average chalone content is very low, and the rate of cell gain exceeds the rate of cell loss. The normal balance can be restored by raising the chalone concentration artificially.

(6) The manner in which this mechanism operates during normal healing and tissue regeneration is examined, and reasons are given for considering that these epidermal reactions are typical of mitotic tissues and of tumours in general.

PART I

It has often been pointed out that an unchanging mass of tissue cells which is constantly replacing itself by mitosis must incorporate a mitotic control mechanism, and that, in theory, the simplest such mechanism would take the form of

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*This article is published in two parts. The references will follow Part 2.

a negative feedback system (Mercer, 1962; Iversen, 1961; Iversen and Bjerknes, 1963; Riley, 1969, 1970). In such a system the basic assumption is that the greater the tissue mass, the higher the concentration of some tissue-specific antimitotic messenger molecule, and the lower the rate of new cell formation (Fig. 1). In 1964 Bullough and Laurence found that epidermis does in fact contain such a substance, which they called the epidermal chalone. Since then 11 other tissues have been found to contain chalone systems with essentially similar characteristics to that of the epidermis (Bullough, 1967, 1971), the best known being the granulocytic system (Rytömaa and Kiviniemi, 1968a).

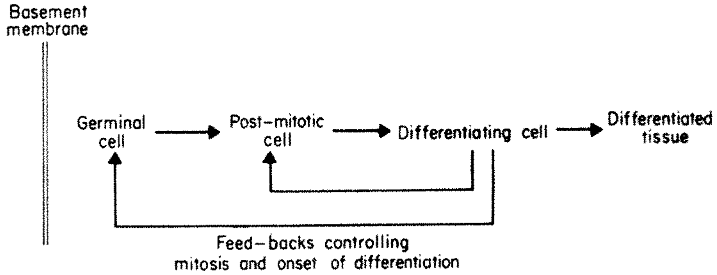

FIG. 1.—Diagram illustrating the negative feedback mechanism theory of mitotic control in epidermis (from Mercer, 1962).

It has, however, recently become clear that a chalone system is not so simple as was originally thought and that tissue mass is not determined solely by the mitotic rate. This article surveys what is known of the way in which epidermal mass, or thickness, is normally maintained, and of the changes that occur in hypoplastic, hyperplastic, and cancerous conditions.

THE EPIDERMAL CHALONE SYSTEM

This system consists, first, of a mechanism for maintaining the appropriate intracellular chalone concentration, and secondly, of a mechanism whereby the epidermal cells, and no others, can respond to the message of this chalone (Bullough, 1967).

The intracellular chalone concentration is determined by the rate of chalone synthesis and by the action of the cell membrane, both in inhibiting chalone loss and in absorbing environmental chalone. Evidently the cell membrane contains an active transport mechanism, and the selectivity of this mechanism may explain the tissue-specificity of the chalone action. The process of mitosis is not tissue-specific, and therefore the antimitotic part of the chalone molecule is itself unlikely to be tissue-specific. It is the remainder of the molecule that may be recognized and accepted by the tissue-specific cell membrane.

On theoretical grounds it has often been suggested that the chalone must be rapidly destroyed by enzyme action within the epidermal cells, since only if the molecular half-life is short can the chalone concentration change quickly enough to provide accurate information on the current state of the epidermis. There is at the moment no evidence that this is so. There is, however, clear evidence that the epidermal chalone, still in its active form, is lost via the blood and the urine (Bullough and Laurence, 1971), and that the fall in the intracellular chalone concentration seen after epidermal damage is probably the result of an increased rate of chalone loss through the cell membrane (Bullough, 1969). Thus the intracellular chalone concentration, and therefore the mitotic rate, may be primarily a function of the state of the active transport mechanism.

The efficiency of the antimitotic action of the epidermal chalone is also increased in the presence of adrenalin and of a glucocorticoid hormone (Bullough and Laurence, 1964a, b).

The mode of action of the adrenalin is still obscure (see Brønstad *et al.*, 1971; Powell *et al.*, 1971); that of the glucocorticoid hormone may be the outcome of induced changes in the cell membrane (Bullough and Laurence, 1968a).

The second part of the epidermal chalone system concerns the nature of the response of the epidermal cells to changes in the chalone concentration. An epidermal cell has 2 obvious alternative activities: it may either synthesize the enzymes needed for cell division, or it may synthesize the enzymes needed for keratin synthesis (it may also perhaps rest indecisively between the 2; Bullough, 1965). The response of an epidermal cell to its chalone is most clearly seen in mouse epidermis after the reduction in the intracellular chalone concentration following epidermal damage (Bullough, 1969). The result is that more basal cells enter mitosis more frequently, and that many of the stratum spinosum cells, which were previously post-mitotic and synthesizing keratin precursors, revert to mitosis. Thus, in the normal epidermis, the chalone must act to inhibit the mitotic activity of the basal cells and to maintain the stratum spinosum cells in their post-mitotic state.

It thus appears that, in the epidermis, the fates of the 2 daughter cells emerging from a mitosis are influenced by the chalone concentration. If this is less than a certain critical level, the cell may enter the mitotic cycle; if it is more than the critical level, the cell may become post-mitotic and begin to synthesize keratin precursors. It is important to note that this selection seems to operate only in cells emerging from mitosis. After a cell has entered the mitotic cycle, a rise in the chalone concentration (or in the stress hormone concentration) merely slows or stops the cycle. The only route into the ageing pathway is via mitosis, and this provides an explanation for the old belief " that cellular transformation during normal differentiation always depends on a quantal mitosis " (*Nature*, 1972). After a cell has entered the ageing pathway, an abnormal fall in the chalone concentration, as after damage, can cause that cell to revert to mitosis.

Evidently, therefore, a low chalone concentration in a cell emerging from mitosis promotes the activity of those genes that initiate and control the mitotic cycle, and, once activated, this genetic programme must pass to completion; and a high chalone concentration in a cell emerging from mitosis promotes the activity of those genes that initiate and control ageing and keratin synthesis, but, in abnormal circumstances, this genetic programme can be terminated (see Bullough, 1965, 1967).

THE POST-MITOTIC CELL LIFE SPAN

The epidermal chalone system also has another less obvious function. Keratin formation is naturally accompanied by cell death, and the chalone system determines both the speed of keratin synthesis and the rate of cell ageing. All post-mitotic cells are dying cells, with a life span that is typical of the tissue concerned; in epidermis this is normally about 7–21 days, according to the type of epidermis

(Epstein and Maibach, 1965; Cameron, 1970). However, with high mitotic activity (low chalone concentration), the epidermal post-mitotic life span is reduced to about 4 days (Scott and Ekel, 1963; Skaeggerstad, 1964; Tables VII, VIII); while, with low mitotic activity (high stress hormone concentration), it may be increased beyond 40 days (Bullough and Ebling, 1952; Table I). The ageing of epidermal cells is clearly not a passive process. The higher the chalone concentration (or the higher the stress hormone concentration), the slower the rate of post-mitotic cell ageing.

An ageing cell passes through at least 2 phases (Fig. 2). In the first (A1), as in the stratum spinosum, the cell synthesizes keratin precursors while retaining the ability to revert to mitosis if, after damage, the chalone concentration falls; in the second (A2), as in the stratum granulosum, the cell loses its ability to revert to mitosis, and degenerative changes are seen in the nucleus (Vaughan and Bernstein, 1971). From studies of other tissues (Bullough, 1967) it appears that, in A1, the genes for tissue function are active, while the genes for mitosis, although silenced by the chalone, retain their potentiality for activity; in A2 all gene activity has finally ceased, and cellular activities are directed by pre-formed messenger RNA. Death comes when this RNA is exhausted, by which time, in the epidermis, keratinization is complete.

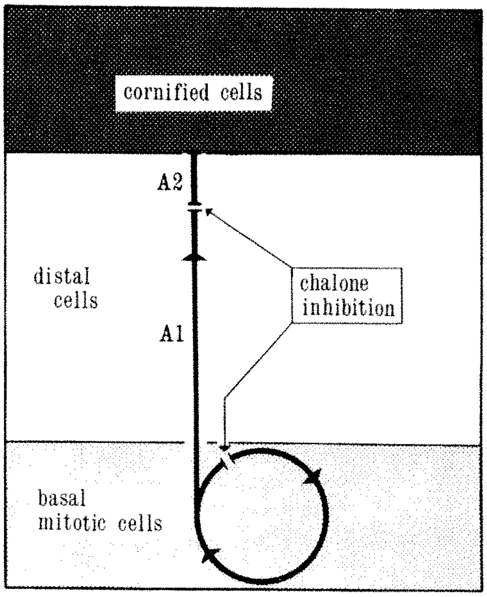

FIG. 2.—Diagram illustrating the flow of cells through the epidermis. The distalpost-mitotic cells pass through 2 phases of ageing (A1 in which they can revert to mitosis, and A2 in which they can not revert).

Thus the epidermal chalone acts to inhibit cell production, to delay cell death, and so to promote the existence of a slowly-ageing post-mitotic cell mass. An extreme situation is seen, for instance, in the liver, in which high chalone efficiency produces an almost static post-mitotic and functional (A1) cell population; in mouse the average potential life span of the liver cells has been estimated at about 3000 days, which is far longer than the life span of the mouse itself (MacDonald, 1961).

THE NEGATIVE FEEDBACK THEORY

The negative feedback theory of mitotic control was originally proposed to explain the way in which tissue or organ mass remains in constant relation to body mass (Fig. 1).

Regarding the epidermis, it is well known that, in any particular area, the thickness (or mass) remains remarkably uniform. The number of epidermal cells beneath a unit area of the stratum corneum is strikingly constant, which must mean that the controlling mechanism is very precise. If the chalone alone is the responsible agent, the basal mitotic cells must be able to respond rapidly to such slight changes in the chalone concentration as would be caused by the local gain or loss of only one or 2 cells. The improbability of this is shown by the dose-response curve, which suggests that the chalone mechanism may be relatively insensitive (Bullough and Laurence, 1964b).

However, an even greater difficulty is posed by the fact that a change in the mitotic rate is always accompanied by a similar change in the rate of post-mitotic cell ageing. A simple negative feedback mechanism could operate efficiently only if, as the mitotic rate changed, the post-mitotic life span remained unchanged.

The conclusion must be that the epidermal chalone mechanism, in the simple form in which it has so far been described, is not adequate to explain the precise control of epidermal thickness that is seen to be achieved.

THE RANGE OF EPIDERMAL MITOTIC ACTIVITY

To analyse the actual nature of the mechanism that controls tissue mass it is useful to consider the whole range of epidermal mitotic activity, from minimal to maximal, and its relationship to epidermal thickness. This range extends from the deep mitotic depression caused by long-continued stress, through normality, to the chronic mitotic stimulus caused by continuous cell damage or by various pathological states, and so the extreme condition seen in a fast-growing epidermal carcinoma. When the evidence is surveyed, it is found that the relationship between mitotic activity and epidermal thickness is not smoothly continuous, but that, at 2 points on the scale of increasing mitotic activity, there are sudden discontinuities in the tissue response (Fig. 3).

Thus the scale is divisible into three phases: Phase 1, which includes normal and hypoplastic epidermis; Phase 2, which includes normal and hyperplastic epidermis; and Phase 3, which includes epidermal carcinomata.

Phase 1. *Normal and Hypoplastic Epidermis*

The body epidermis of mouse and rat, as well as hypoplastic human epidermis, all belong to Phase 1, which is characterized by a relatively low mitotic rate (in mouse ear about 3% basal epidermal cells enter mitosis daily; Bullough, 1969), a relatively thin epidermis (usually only 2 obvious cell layers, although more in fact exist; see Mackenzie, 1970), and a flat basal cell layer (ratio inner epidermal surface : outer epidermal surface = 1; Tosti *et al.*, 1959).

TABLE I.—*Relation between Mitotic Rate, Tissue Mass and Estimated Post-mitotic Life Span in the Epidermis and Sebaceous Glands of Adult Male Mice*

Epidermis			Sebaceous glands		
Mitotic rate*	Thickness (μm)*	Estimated cell life span	Mitotic rate*	Gland mass (no. of cells)*	Estimated cell life span
4·5±0·34	16·8±0·2	c. 45 days	0·18±0·05	350·4±31·8	c. × 4·5 normal
4·6±0·23	16·0±0·7	c. 45 days	0·24±0·05	360·6±23·7	c. × 4 normal
7·6±0·52	16·9±0·6	c. 26 days	0·52±0·08	385·6± 6·4	c. × 2 normal
16·6±1·16	16·4±0·6	c. 12 days	0·94±0·10	365·4±23·3	Normal
19·5±1·46	16·4±0·2	c. 10 days†	0·88±0·08	387·0±31·8	Normal

* From Bullough and Ebling (1952): N = 5 throughout.
† From Cameron (1970): actually 7–14 days.

However, the most important characteristic of Phase 1 epidermis is that, when the mitotic rate changes, the epidermal thickness is not affected. Table I shows the effects of long-continued stress (caused by restricted diets) on epidermal mitotic activity and thickness in mice. Even a mitotic rate of less than 25% normal maintained for 4 weeks causes no change in epidermal thickness, and also incidentally no change in sebaceous gland mass. Table II shows similar results with rat epidermis after castration or testosterone treatment. Mouse liver reacts in a similar way (Table III: the cirrhotic livers had no greater mass than normal livers unless they accumulated fat).

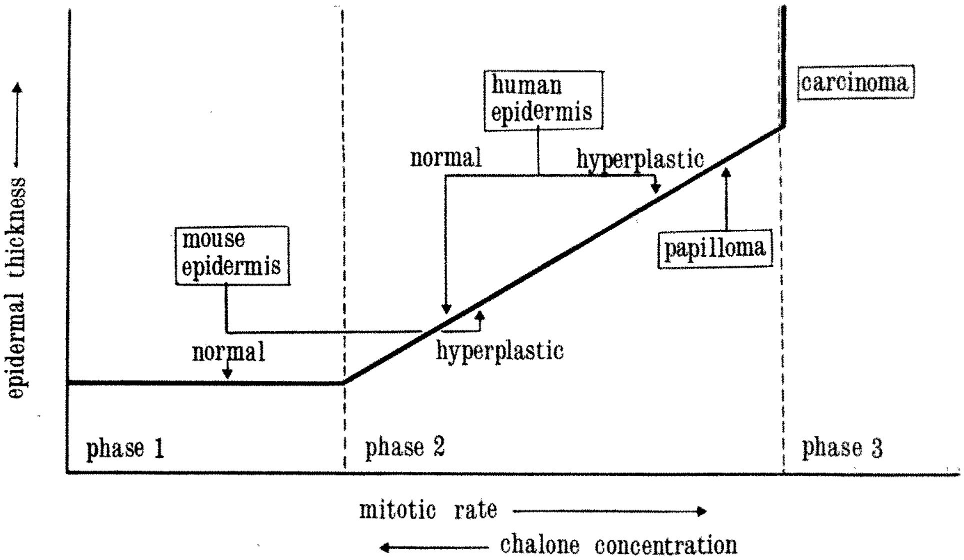

FIG. 3.—Relation between the mitotic rate and the thickness of the epidermis.

TABLE II.—*Relation between Mitotic Rate, Tissue Mass and Post-mitotic Life Span in the Epidermis and Sebaceous Glands of Adult Male Rats**

	Epidermis			Sebaceous glands		
Treatment	Mitotic rate	Thickness (μm)	Estimated cell life span	Mitotic rate	Gland mass (nos. of cells)	Estimated cell life span
Castrated	31·9±7·4	20·7±1·0	c. 28 days	34·1±5·5	205·5±23·7	6–7 days
Normal	47·0±6·4	18·8±1·2	c. 19 days†	73·6±9·9	253·3±19·3	3–8 days
Castrated and injected with testosterone	63·1±4·9	19·1±1·1	c. 14 days	124·4±14·8	322·4±42·0	2–6 days

* From Ebling (1957a) : N = 8.
† From Bertalanffy (1957).

Thus, throughout Phase 1 any changes in the mitotic rate are immediately counterbalanced by changes in the rate of post-mitotic cell ageing. The mitotic rate can be defined as the number of mitoses occurring per cent of the basal epidermal cells per day, and this is easily measured. The rate of post-mitotic ageing can be defined in terms of the number of days taken by an epidermal cell to pass from its last mitosis to its death in the base of the stratum corneum, and this cannot be so accurately determined (the figures given in the various tables are only approximate).

TABLE III.—*Relation between Mitotic Rate and Post-mitotic Life Span in Rat Liver**

Condition of liver	Mass of liver	Mitotic cells per cent	Estimated post-mitotic life span
Normal	Normal	0·266%	376 days
Cirrhotic	Unchanged†	3·8%	26 days

* From MacDonald (1961).
† Liver enlarged only if fatty.

Although the ratio rate (mitosis) : rate (ageing) is a constant, the value of this constant is not the same in all types of epidermis. Table IV shows how the ear and back epidermis of the guinea-pig have the same mitotic rates but different thicknesses, and how these are related to the different times taken for post-mitotic cell ageing.

TABLE IV.—*Relation between Labelling Index, Tissue Mass and Post-mitotic Life Span in Normal Guinea-pig Epidermis**

Body region	^{3}H-T labelled basal cells/mm	Epidermal thickness	Post-mitotic life span
Ear epidermis	9·6±3·4	1·90±0·18	c. 14 days
Back epidermis	10·4±2·6	1·21±0·12	c. 8 days

* From Christophers and Petzold (1969); N = 8 throughout.

Thus, the thickness of any particular type of Phase 1 epidermis is determined first, by the fact that the chalone concentration is high enough to place it in Phase 1, and secondly by its own inherent rate of post-mitotic cell ageing.

Throughout Phase 1 changes in the epidermal mitotic rate are confined to the basal layer, but the cells of this layer are not homogeneous (Iversen *et al.*, 1968): some are involved in the mitotic cycle and some are already in the early post-mitotic phase (early A1 in Fig. 2). In these latter cells, in mouse ear epidermis, the early synthesis of keratin precursors has progressed so far that the cells can be stained diagnostically and their behaviour determined (Christophers, 1971; Fig. 4). The mitotic cells usually divide in such a plane that the 2 daughter cells remain basal (Bullough and Laurence, 1964a), and the pressure so generated is then relieved by the displacement distally of a closely adjacent early post-mitotic cell. This suggests that, in early A1, the attachment of the cell to the dermis is weakened; in the mitotic cells it is evidently strong.

When, from a low level in Phase 1, the epidermal mitotic rate rises, this is due partly to a faster mitotic cycle (both G1 and the mitotic phases shorten) and partly to an increasing proportion of the basal cells becoming involved in the mitotic cycle (with a consequent decreasing proportion being post-mitotic). The importance to the mitotic rate of the proportion of cells that are in the mitotic cycle has been emphasized by Epifanova (1966) (Table V).

The fact that, the weaker the chalone action the greater is the proportion of basal cells that become involved in the mitotic cycle, suggests that these basal cells must vary in their chalone content. Thus, as the average chalone (or stress

TABLE V.—*Relation between Mitotic Rate, Mitotic Duration, Inter-mitotic Interval and Proportion of Cells in Mitotic Cycle in Mouse Uterus**

Treatment	Mitotic rate	Duration of mitosis + G_1 = cycle	Proportion of cells in cycle
Ovariectomized	2·3 ± 0·3‰	10·5 h + 31·5 h = 42 h	8·3%
Two injections of oestrone	9·8 ± 0·7‰	7·5 h + 18·5 h = 26 h	27·9%

* From Epifanova (1966).

hormone) concentration falls, those basal post-mitotic cells that had the lowest chalone content (presumably the youngest post-mitotic cells) are probably the first to revert to mitosis.

The fact that, the weaker the chalone action the shorter is the intermitotic interval, suggests that, even in the mitotic cells, with their subthreshold chalone concentrations, the chalone must continue to exert a restraining influence on the mitotic cycle in proportion to its concentration.

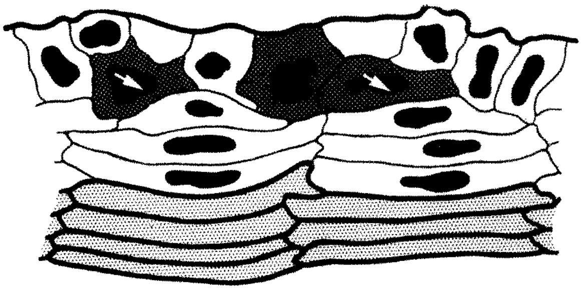

FIG. 4.—Normal mouse epidermis. The basal layer contains both mitotic (white) and post-mitotic (stippled) cells (from a preparation by Dr E. Christophers).

The fact that, the weaker the chalone action the faster the post-mitotic cells age and die, indicates clearly how, in these cells, with their suprathreshold concentrations, the chalone must also act to inhibit cell death in proportion to its concentration.

Conclusions.—The Phase 1 type of epidermis has the following characteristics:

(1) The mitotic rate is relatively low. The daughter cells of each mitosis normally remain basal, and the consequent pressure forces out one closely adjacent post-mitotic cell. Such a cell does not leave the basal layer unless it is so forced.

(2) The lower the average chalone concentration in the epidermis, the more basal cells remain mitotic. The lower the average chalone concentration within the mitotic cells, the faster these cells age.

(3) The ratio between the mitotic rate and the rate at which the post-mitotic cells age is a constant, and consequently a changing mitotic rate has no effect on epidermal thickness.

(4) The thickness of Phase 1 epidermis is determined solely by the time taken for the post-mitotic cells to age and die.

Phase 2: Normal and Hyperplastic Epidermis

The epidermis in Phase 2 has a relatively high mitotic rate (in normal human epidermis > 10% of basal cells enter mitosis per day; Heenan and Galand, 1971), it is relatively thick (more than 2 obvious cell layers), and it has a folded (or doubled) basal layer (when folded, ratio inner epidermal surface: outer epidermal surface > 1; Tosti *et al.*, 1959). These features are seen normally in the epidermis, for instance, of guinea-pig (Christophers, 1969), pig (Winter, 1966), and man (Tosti *et al.*, 1959), and abnormally in any hyperplastic epidermis.

However, the most significant characteristic of Phase 2 epidermis is that, the higher the mitotic rate, the thicker the epidermis (Christophers, 1969) (Tables VI and VII and Fig. 3).

TABLE VI.—*Relation between Rate of Cell Division and Thickness of Normal Guinea-pig Epidermis**

^{3}H-T labelled basal cells/mm	Average epidermal thickness (μm)	Number of animals
10·6±1·16	33·7±1·85	4
15·6±0·47	38·9±0·96	7
19·1±0·55	42·6±1·07	7
22·6±0·30	43·2±2·23	8
29·8±0·49	53·2±3·84	6

* Calculated from data provided by Dr E. Christophers.

TABLE VII.—*Relation between Mitotic Rate, Epidermal Thickness and Post-mitotic Life Span in Guinea-pig Epidermis* and in Psoriatic Human Epidermis*†

Treatment or condition	Mitotic rate	Increase in epidermal thickness	Post-mitotic life span
Guinea-pig epidermis:			
Untreated	Normal	×1	c. 14 days
Petroleum jelly 2 days	Increased	×1·26	9–10 days
Petroleum jelly 8 days	Increased	×1·9	9–10 days
Vitamin A 11 days	High	×3·5	c. 3 days
Hexadecane 2 days	×10–15	×2·2	c. 3 days
Hexadecane 7 days	×10–15	×4·4	c. 3 days
Human epidermis:			
Normal	Normal	×1	c. 27 days
Psoriasis	High	×4·15	2–4 days

* From Christophers (1970) and Christophers and Braun-Falco (1970).
† From Scott and Ekel (1963).

Phase 1 epidermis is transformed into Phase 2 epidermis when, following damage, its mitotic rate rises above a certain level; similarly Phase 1 sebaceous glands enter Phase 2 when their mitotic rate exceeds a certain level after testosterone treatment (Table II).

In epidermis at the breakpoint from Phase 1 to Phase 2 the chalone content of the basal cells evidently falls to such a level that all these cells enter the mitotic cycle. The outcome of one mitosis is then one extra basal cell; there is no basal post-mitotic cell remaining to be pushed out (Fig. 5; compare Fig. 4). Thus, in the basal layer the pressure rises and the layer begins to fold (in mouse it begins to double). The result is an increasing *number* of basal mitotic cells per unit area of skin; and, although the rate of post-mitotic ageing also increases, this naturally leads to an increasing *number* of distal post-mitotic cells per unit area of the skin (Fig. 6). Thus the epidermis thickens in proportion to the mitotic rate.

Of course, the breakpoint also exists in reverse from Phase 2 to Phase 1, as when hyperplastic mouse epidermis reverts to normality, or when the mitotic activity of human epidermis is reduced in senility (Tosti *et al.*, 1959, 1969). Senile human epidermis is termed hypoplastic and, being in Phase 1, it cannot become any thinner; mouse epidermis, being naturally in Phase 1, cannot become hypoplastic.

TABLE VIII.—*Relation between Mitotic Rate and Post-mitotic Life Span in Human Epidermis with Ichthyosiform Dermatoses**

Condition of epidermis	Labelled cells/cm surface line ±S	Post-mitotic life span	Number of cases
Normal controls	86±21	12–14 days	18
Ichthyosis vulgaris	70±7	10–14 days	4
Lamellar ichthyosis	231±36	4–5 days	3
Epidermolytic hyperkeratosis	294±85	4 days	3

* From Frost *et al.* (1966): S=standard deviation.

The components of a rising mitotic rate in Phase 2 are as follows: a progressive shortening of the mitotic cycle (at the extreme it may perhaps approach 12 hours, as in hair bulbs; Bullough and Laurence, 1958), an increasing number of mitotic cells per unit area of skin, due to the increasing folding of the basal layer (in man) the maximum value for the ratio inner epidermal surface: outer epidermal surface is about 4·5; Tosti *et al.*, 1959, 1969), and the progressive disappearance of the diurnal mitotic cycle. The cycle fades away as the chalone concentration falls to such low levels that the stress hormones, in their normal concentrations, find too little chalone with which to co-operate (Bullough and Laurence, 1961, 1964b).

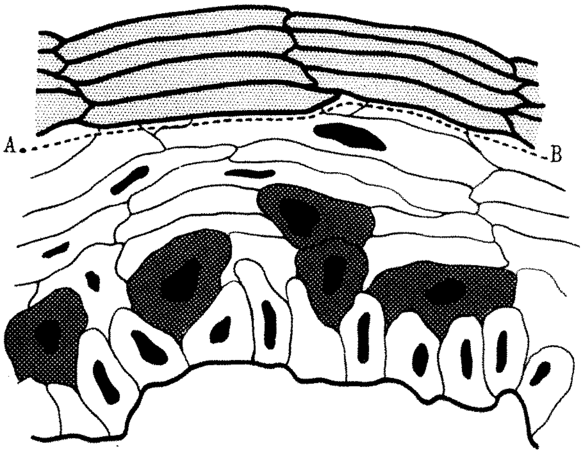

FIG. 5.—Mouse ear epidermis at onset of hyperplasia. The basal post-mitotic cells (stippled) have all been pushed distally, and the distal cells are becoming disordered. Above A–B the ordered arrangement of the squames has not yet been lost (from a preparation by Dr E. Christophers).

As the mitotic rate rises, so also does the rate of postmitotic ageing (Tables VII, VIII). This shows clearly that *all* the epidermal cells are responding and therefore that the average chalone content of the *whole* epidermis is progressively reduced (Fig. 7). Each point of balance in Phase 2 is achieved, not because the thicker epidermis restores the average chalone content to " normal " (as would be expected in a negative feedback system), but because a limit is imposed by the length of the post-mitotic life span that is typical of that point on the Phase 2 scale.

In the epidermis the upper end of Phase 2 includes a range of pathological conditions from the generalized thickening of psoriasis to the localized thickening of static papillomata and other similar tumours. In all of these conditions the rate of cell loss is adequate to balance the rate of cell gain.

Conclusions.—The Phase 2 type of epidermis has the following characteristics:

(1) The mitotic rate is relatively high, and all the basal cells are mitotic; no basal post-mitotic cells remain to be driven out.

(2) Thus, throughout Phase 2, the greater the mitotic rate, the greater the pressure in the basal layer, the greater the degree of folding (or doubling) of this

layer, the greater the number of cells (mitotic and post-mitotic) per unit area of the skin, and the thicker the epidermis.

(3) However, the higher the mitotic rate, the higher the rate of post-mitotic ageing. Consequently, at any point in Phase 2, the epidermal thickness is limited by the length of the post-mitotic ageing process that is typical of that particular point.

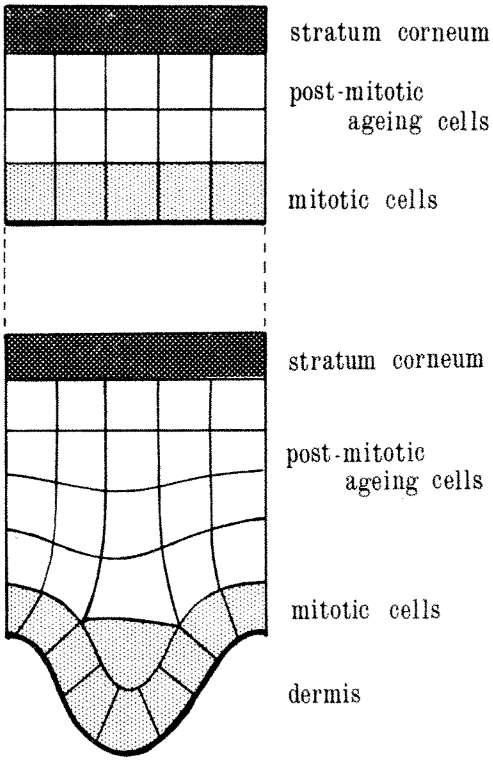

FIG. 6.—Diagrammatic representation of epidermis. Above is Phase 1 epidermis, in which a changing mitotic rate does not affect thickness. Below is Phase 2 epidermis, in which an increasing mitotic rate causes increasing pressure, which results in the increasing folding of the basal layer.

EPIDERMAL HOMEOSTASIS

It is now possible to understand how epidermal thickness is so precisely maintained. The 2 vital factors are the chalone content of the cells, which determines their position in Phase 1 or Phase 2, and the time taken by the post-mitotic cells to age and die, which is peculiar to each type of epidermis and which changes in such a way that rate (mitosis): rate (ageing) remains a constant.

In normal circumstances the average chalone content of each region of the epidermis evidently remains unchanged, although, within each region of the cell, chalone content may not be uniform. As a general rule, it seems that the older a post-mitotic cell becomes the more chalone it contains: thus, while gentle massage will stimulate extra mitoses in the basal cell layer, it requires considerable damage, and a fall of at least 50% in the intracellular chalone concentration, to bring the cells of the stratum spinosum back into mitosis (Bullough and Laurence, 1964a). The older post-mitotic cells may have a higher rate of chalone secretion, a less permeable cell membrane, or both.

Epidermal mitotic activity varies widely from hour to hour with the varying concentration of the 2 stress hormones; this is the diurnal mitotic cycle. Thus the normal mitotic rate (and probably also the normal rate of post-mitotic ageing) is the average of a wide diurnal fluctuation.

Chronic mitotic depression.—The 2 commonest natural causes of chronic epidermal mitotic depression are long-continued stress and old age. It is known that, in such circumstances, mouse epidermis, being in Phase 1, does not change its mass, and even the rate of loss of the outer squames of the stratum corneum seems to be reduced. Less is known of the reactions of human epidermis. However, Shanon (1970) has noted that in men " under severe stress in concentration camps " psoriasis was " ameliorated or disappeared entirely ", which suggests that the hyperplasia must have been reduced; and it is also well known that senility leads to hypoplasia (Tosti *et al.*, 1959, 1969) as the epidermis moves into Phase 1.

Mitotic stimulation.—Hyperplasia is the natural response to any form of epidermal damage. It may be transient when it leads to the regeneration of the normal epidermal thickness, or it may be chronic when it leads to epidermal thickening.

The sequence of events after transient damage is most clearly illustrated by the response of mouse epidermis to the removal of the stratum corneum and of the distal cell layers. The fall in the chalone content within the remaining basal cells then leads quickly to a raised mitotic rate (which can be prevented by chalone treatment; Frankfurt, 1971). The new post-mitotic cells so produced have, in theory, a reduced life expectancy of only some 4 or 5 days, but, before this time elapses, the epidermal thickness approaches normality, the average chalone concentration rises, the mitotic rate falls, and the post-mitotic cells acquire a longer life span. The precise epidermal thickness is then fixed by the characteristic life span of the post-mitotic cells.

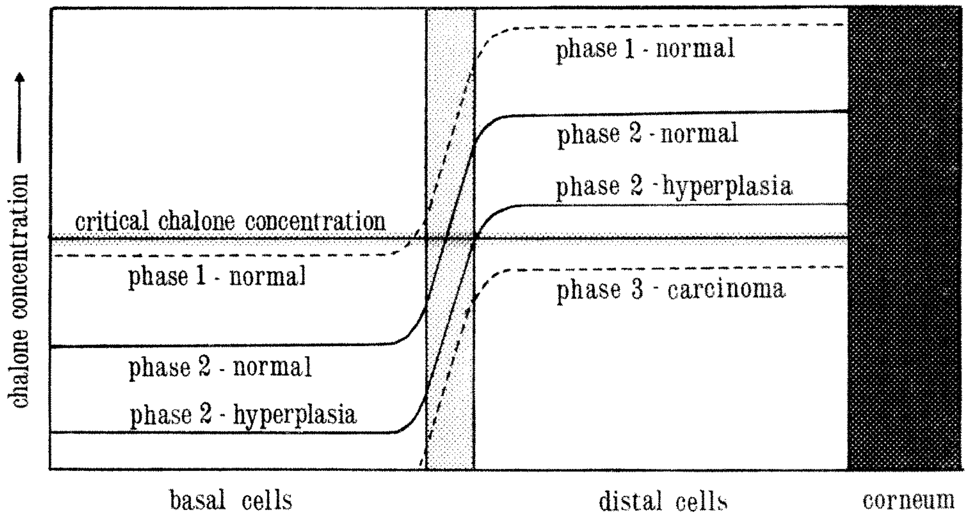

FIG. 7.—Diagram of epidermis, showing the chalone concentrations typical of Phases 1-3.

In human epidermis the response is the same except that, with a naturally higher mitotic rate (lower chalone content), the epidermis returns to its normal position in Phase 2.

The response to a simple wound also follows the same pattern except that it is confined to a narrow zone around the wound edge (Bullough and Laurence, 1960). Within this zone the basal cells, already abnormally crowded by the outward contraction of the wound edges, increase their mitotic rate, and some of the distal cells also revert to mitosis. Thus, along the wound edge there is an abnormally high number of mitotic cells per unit area, and this leads rapidly to an abnormal production of post-mitotic cells, which, again in theory, have a reduced life span of only a few days. However, epidermal continuity is usually re-established so quickly that, as the mitotic rate falls with the rising chalone concentration, the new post-mitotic cells acquire their usual longer life span. Undue epidermal thickening is avoided partly, in theory, because of the reduced post-mitotic life span and partly, in practice, because so many of the cells of the wound edge move out over the wound cavity to resurface it.

If the epidermal damage is chronic, Phase 1 epidermis (e.g. mouse) moves into Phase 2, while Phase 2 epidermis (e.g. man) rises to a higher level in Phase 2. Both types of epidermis thicken to the point determined by the increased rate of post-mitotic ageing.

(To be concluded.)

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ELECTRONMICROSCOPIC STUDY OF HYPOPIGMENTED LESIONS IN LEPROSY

A PRELIMINARY REPORT

C. K. JOB, AMMU NAYAR AND J. S. NARAYANAN

Summary.—The electronmicroscopic appearance of the epidermis in 2 cases of indeterminate leprosy was studied. There was a significant decrease in the number of melanocytes and several of those present showed signs of depressed activity and atrophy. Langerhans cells were increased in number and seemed to replace the melanocytes. Further study is indicated to elucidate the pathogenesis of melanocyte inactivity and reduction in number.

HYPOPIGMENTATION of the skin is characteristic of all forms of leprosy (Cochrane, 1964) and is perhaps one of the earliest detectable signs of the disease. Cutaneous tuberculoid lesions with complete loss of tactile sensitivity, and macular skin lesions of indeterminate leprosy without any demonstrable loss of sensations, often manifest as hypomelanotic patches. In bacillated lesions of lepromatous leprosy, hypopigmented lesions, although present, are not so striking.

Patchy hypopigmentation can be attributed to a localized defect in melanogenesis. In an earlier study melanocytes from hypopigmented skins from leprosy patients were examined, using dopa-stained epidermal sheets. Skin from the apparently normal area on the contralateral side of the same patient was studied as a control. It was found that there was a significant reduction in melanocyte population and depression of melanocyte function localized to the leprous lesions (Nayar and Job, 1970). The present electronmicroscopic study of the epidermis from the hypopigmented cutaneous leprosy lesions was undertaken to elucidate further the nature of the pathological process producing the defect in melanogenesis.

MATERIAL AND METHODS

Biopsies of early skin lesions, clinically diagnosed as indeterminate leprosy, were obtained from 2 patients among the many gathered through surveys of contacts of leprosy patients. Control specimens from both patients were obtained from the same region on the contralateral side, showing no obvious lesions. The biopsies were divided into two, and one piece was fixed in Formol-Zenker solution, and processed. Paraffin sections were made and studied to confirm the diagnosis. The other piece was cut into 1 mm cubes, fixed in glutaraldehyde, post-fixed in 1% osmic acid, processed in graded alcohol and embedded in Araldite. Ultrathin sections were cut, using a Cambridge Huxley microtome, stained with uranyl acetate and lead citrate and examined using a Philips EM-200 electron microscope.

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RESULTS

Histologically the skin lesions from both patients showed small focal collections of round cells around skin appendages, including nerve bundles. In one case 2 acid-fast bacilli were seen inside a nerve bundle, and one bacillus was seen just beneath the epidermis. The diagnosis of indeterminate leprosy was thus histopathologically confirmed in both patients.

The ultrastructural appearance of normal melanocytes in the epidermis is well known (Zelickson, 1967), and they are easily distinguished from keratinocytes. Melanocytes are dendritic and are very often located above the basement membrane at the basal layer. Desmosomes are absent. The nuclei are round and have a smooth, clear, double nuclear membrane. Mitochondria are numerous and are present throughout the cytoplasm. Endoplasmic reticulum is also abundant and distributed throughout the cytoplasm. The cytoplasm is clear, due to the absence of keratin fibrils. The Golgi region is well developed, and melanin granules in varying stages of maturity are present. In the control specimens from both patients the melanocytes of the basal layer showed all these characteristic features (Fig. 1). Their appearance and distribution were as in normal epidermis.

The Langerhans cells are also readily identified by their special features (Zelickson, 1965). They, too, have clear cytoplasm and lack desmosomes. Although mitochondria are abundant and the Golgi zone is well developed, melanosomes are ordinarily absent. Further, this cell has a characteristic granule which, in longitudinal sections, consists of rod-shaped profiles having a striated central lamella. The nucleus is convoluted. In the control specimens from both patients, Langerhans cells were present in the suprabasal region and in the basal layer.

The appearance of the epidermis from the hypopigmented indeterminate leprosy lesions from the 2 patients was almost identical. Normal melanocytes containing melanosomes in various stages of maturation were present (Fig. 2), although there was an appreciable reduction in their number. The cytoplasm of a few melanocytes was retracted from the surrounding keratinocytes and the space thus provided was filled by electron dense granular material (Fig. 3). Some of the melanocytes were vacuolated and atrophic, with fewer mitochondria, and dilated endoplasmic reticulum (Fig. 4). In some there was a significant reduction in endoplasmic reticulum and premelanosomes, and the Golgi complex was hardly seen. Folding and reduplication of epidermal basement membrane adjoining several melanocytes was present (Fig. 5). There were typical Langerhans cells in both the basal and suprabasal layers, containing the characteristic granules (Fig. 6). An appreciable increase in their number, as compared with the control specimen, was noted. Further, a few Langerhans cells with indented or convoluted nuclei were apparently atrophic and inactive (Fig. 7), and the cytoplasm of some of these atrophic cells was retracted (Fig. 8). The retraction of the cytoplasm could be a preparation artifact. However, it must be pointed out that the surrounding cells in the same section were well fixed and showed a normal appearance.

In the subepidermal region quite close to the basement membrane a few Schwann cell processes containing axons and occasional *M. leprae* in various stages of degeneration were seen (Fig. 9). In some, the Schwann cell cytoplasm contained large vacuoles, and the Schwann processes showed marked thickening and reduplication of the basement membrane. Similar reduplication was seen in the basement membrane of the melanocytes.

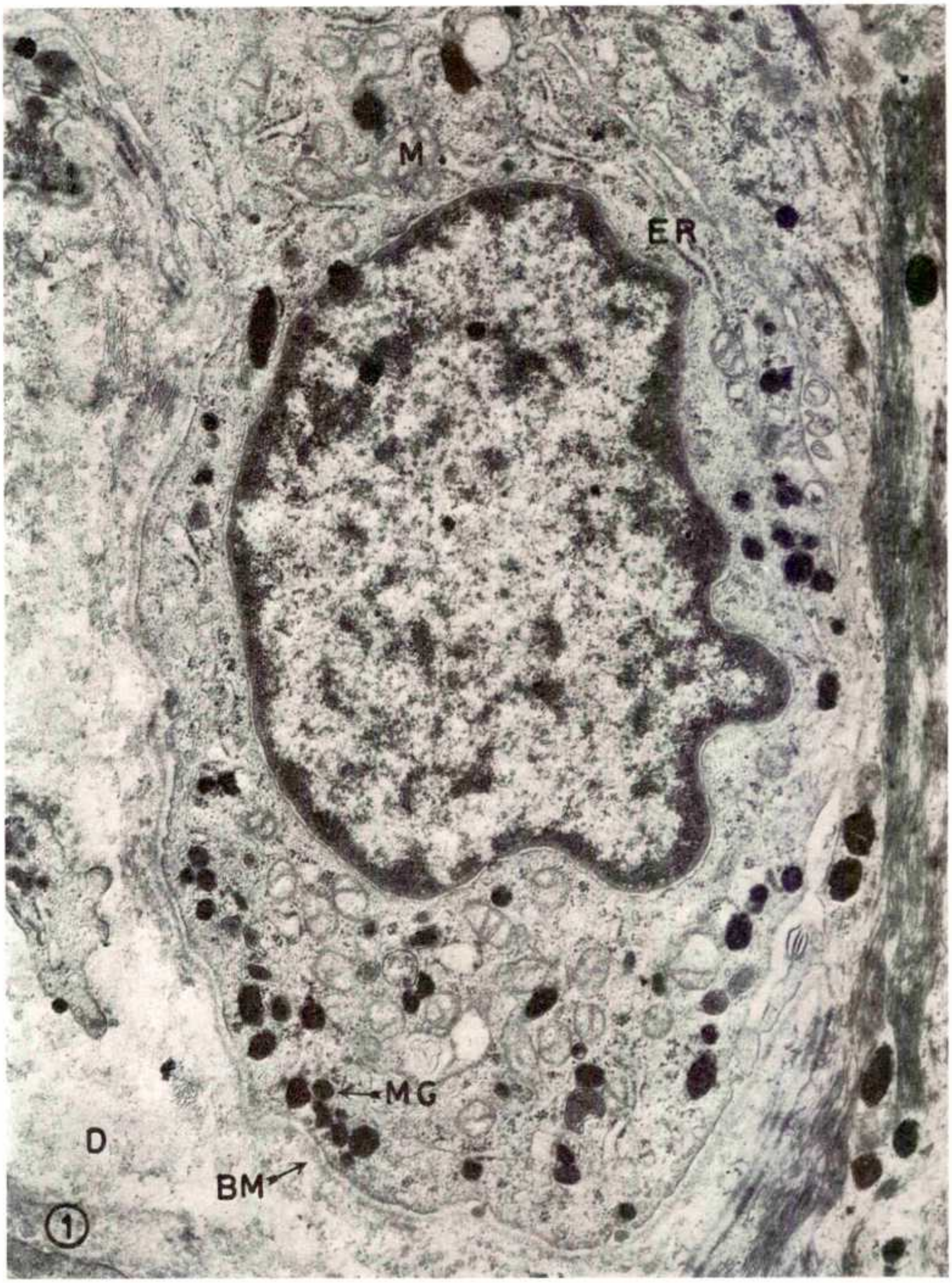

FIG. 1.—A melanocyte in normal epidermis, located just above the basement membrane (BM) bulging into the dermis (D). Note the abundance of mitochondria (M) and well developed rough endoplasmic reticulum (ER). Numerous mature melanin granules (MG) are present. (× 21,000)

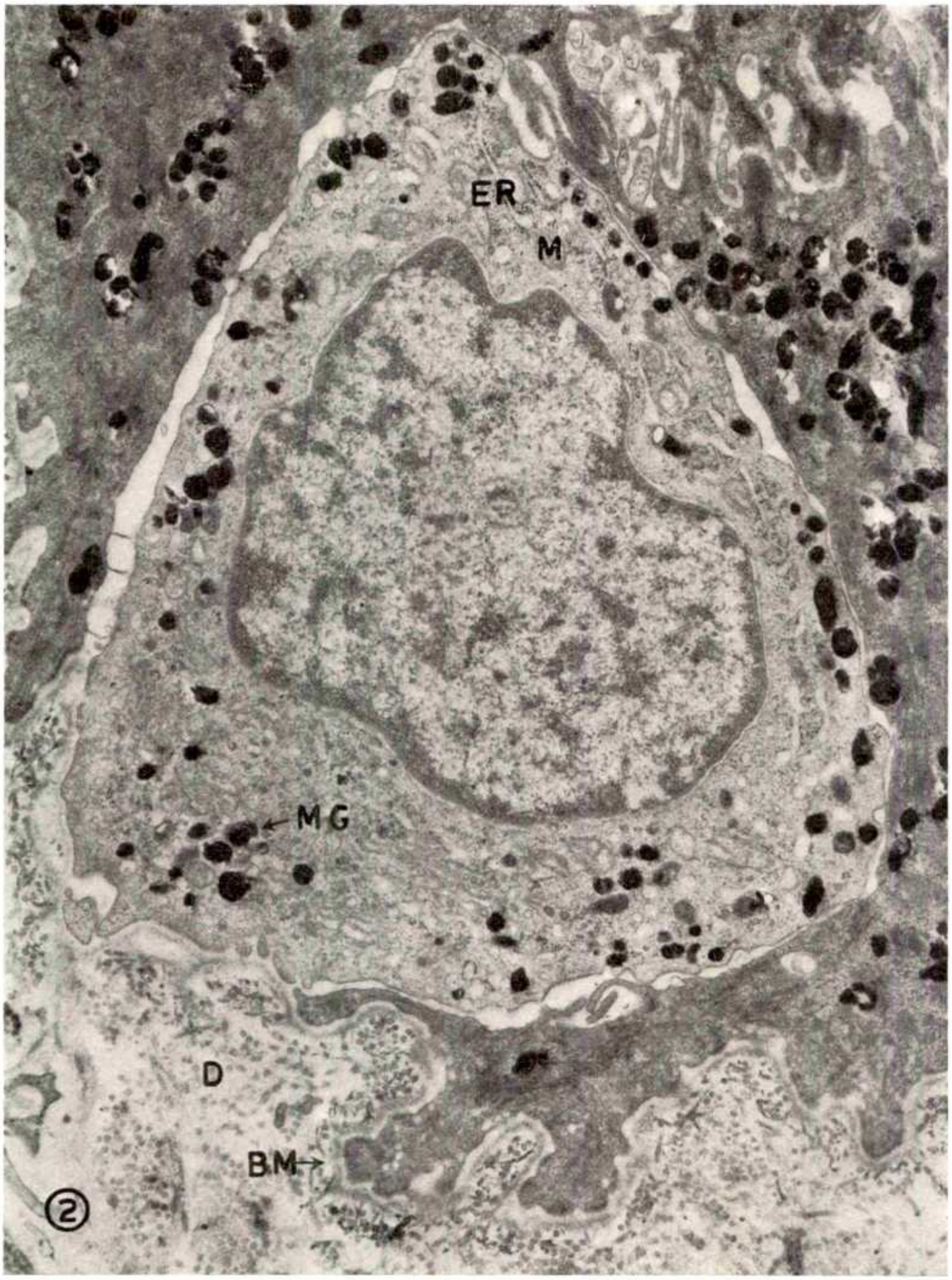

FIG. 2.—A normal melanocyte in the hypopigmented lesion in leprosy, located just above the basement membrane (BM). Mitochondria (M), rough endoplasmic reticulum (ER) and melanin granules (MG) are uniformly distributed in the cytoplasm. (× 18,000)

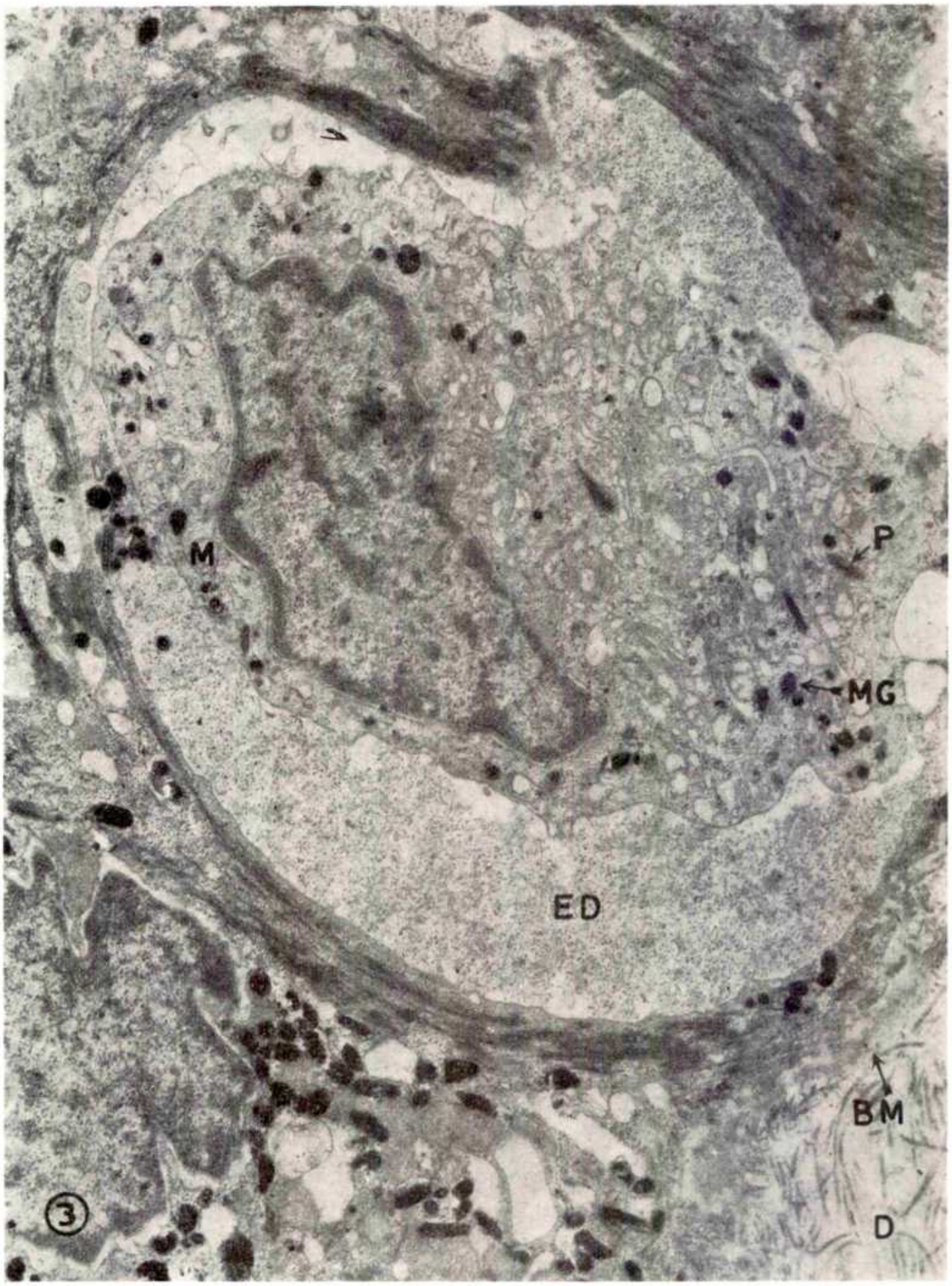

FIG. 3.—A melanocyte in the hypopigmented leprosy lesion. Note the shrinkage of cytoplasm and the electron dense granular material (ED) filling up this area. Although there are numerous mitochondria (M), melanin granules (MG) are less than in normal, indicating reduced melanogenic activity. However, a few premelanosomes (P) were present. (× 15,000)

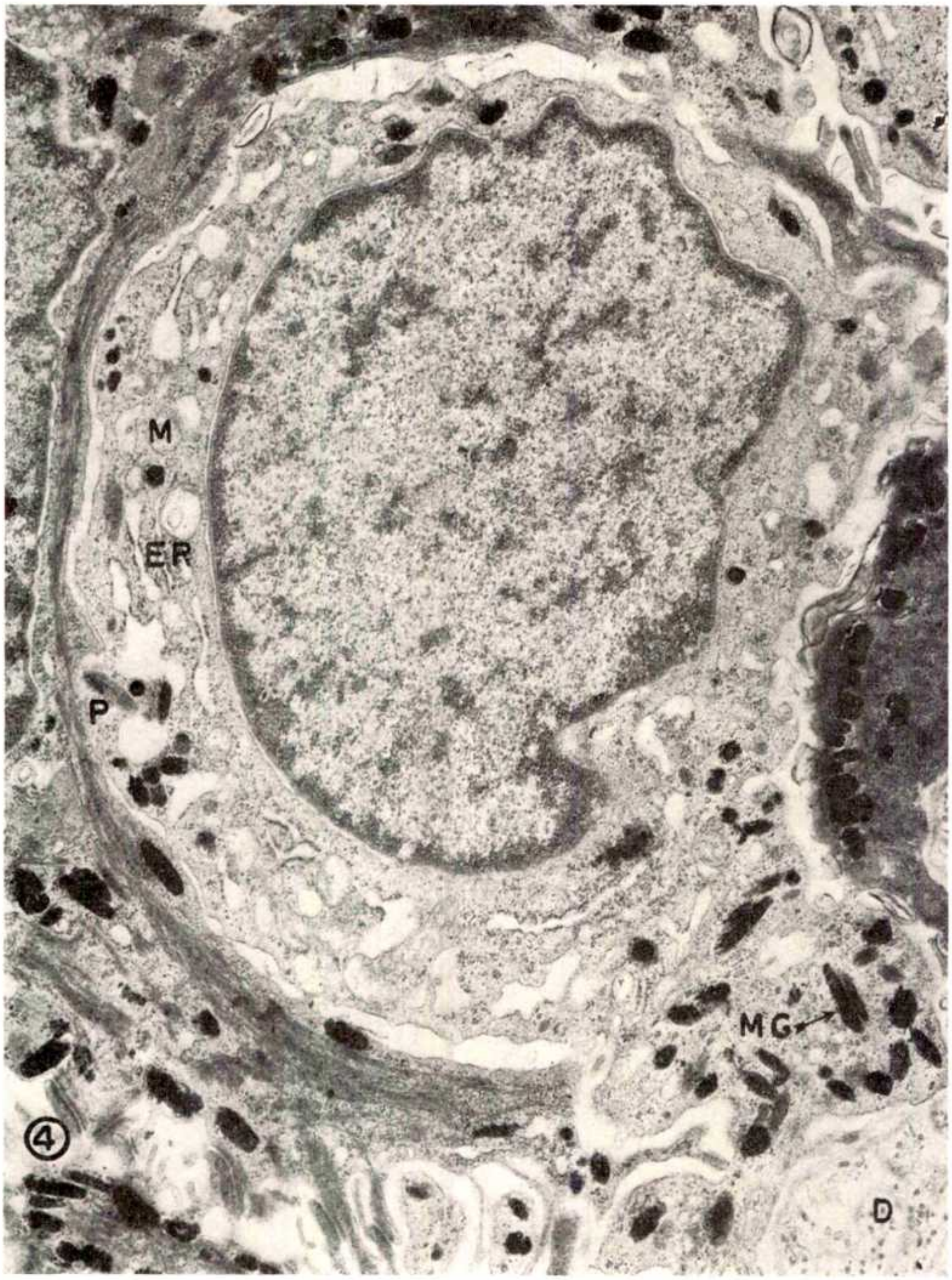

FIG. 4.—An atrophic melanocyte from the lesion, showing significantly reduced number of mitochondria (M). The cytoplasm is vacuolated and the rough endoplasmic reticulum (ER) shows marked dilatation. A few premelanosomes (P) are present. (× 18,000)

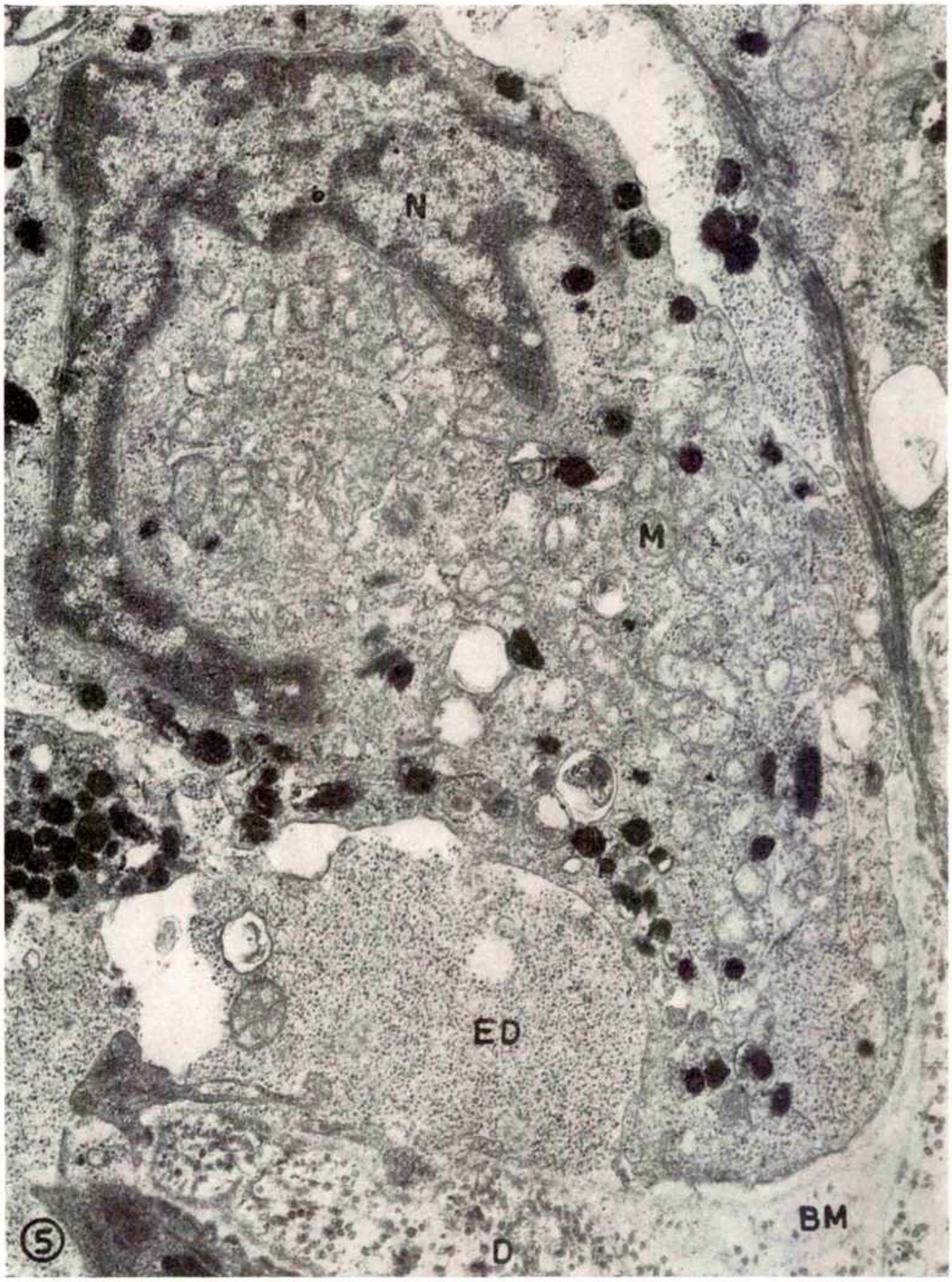

FIG. 5.—A melanocyte from the lesion, with an indented nucleus (N). The mitochondrial (M) proliferation is marked, but rough endoplasmic reticulum is much less. The cytoplasm is retracted and this space is filled with electron dense granular material (ED). Only a few premelanosomes are seen. Note the reduplication of the basement membrane (BM) adjoining the melanocyte. (× 24,000)

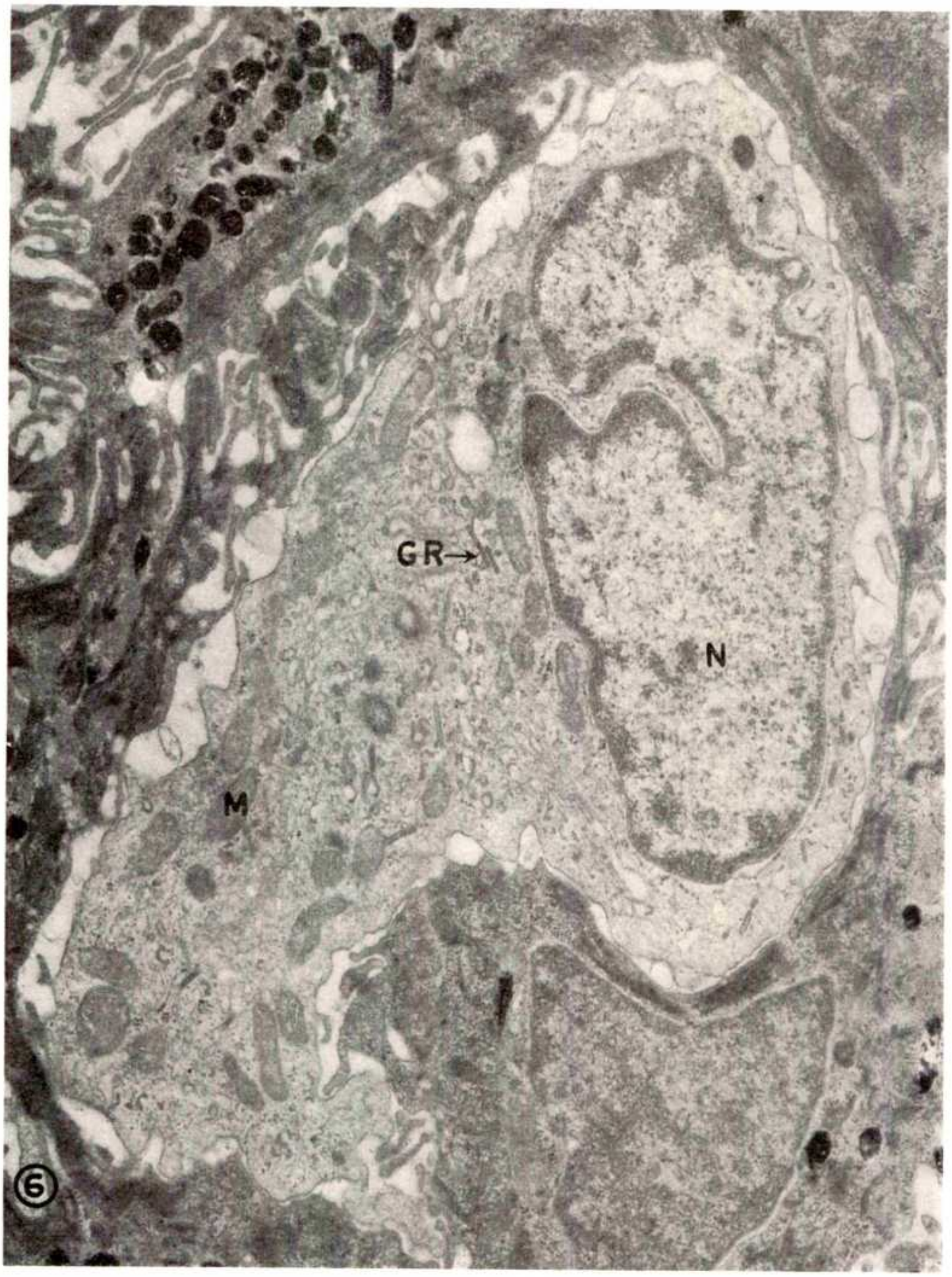

FIG. 6.—A typical active Langerhans cell from the hypopigmented lesion in leprosy. Note the clear cytoplasm, the characteristic granules (GR) and indentation of the nucleus (N). (× 21,000)

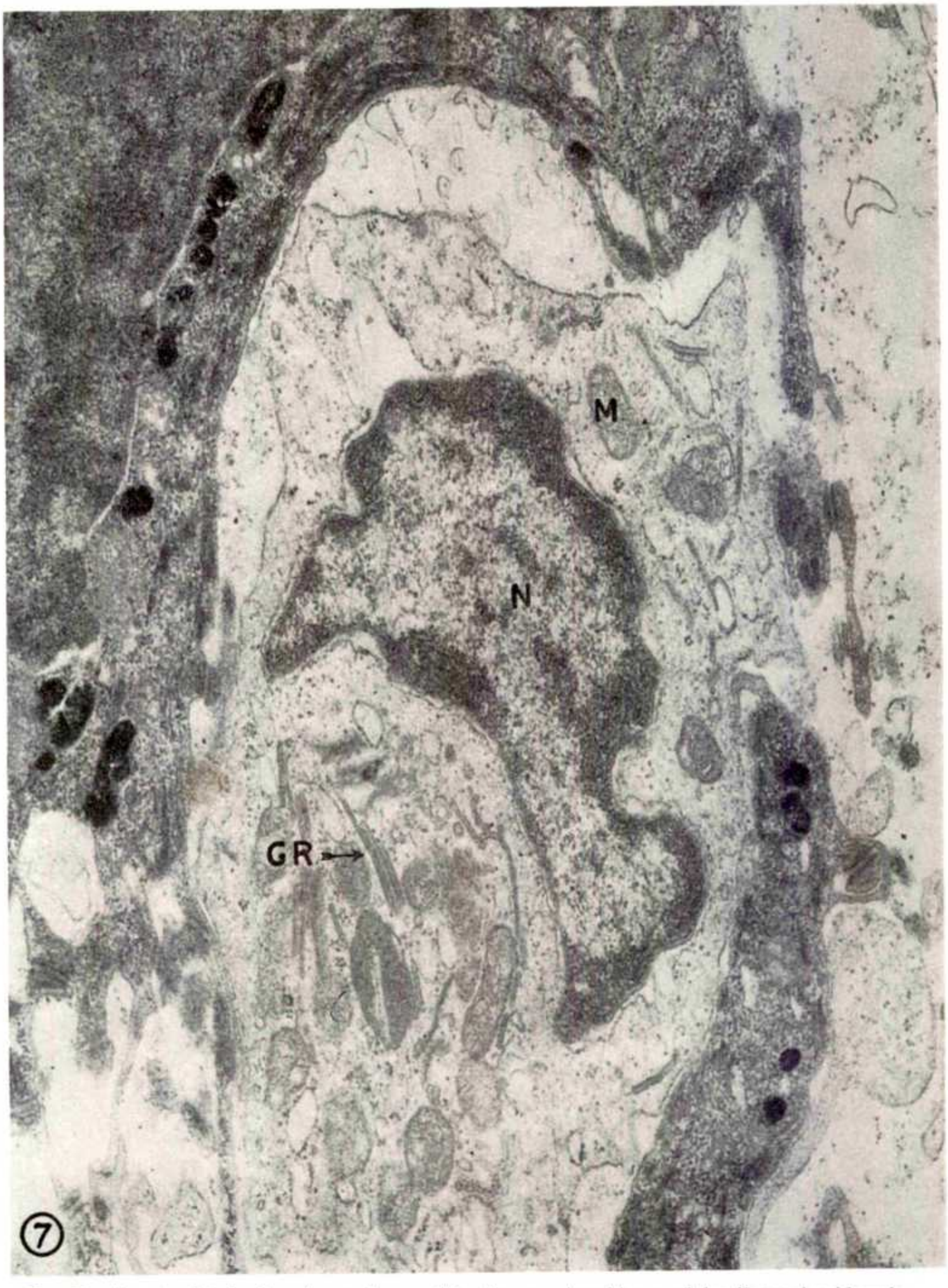

FIG. 7.—An atrophic-looking Langerhans cell in the suprabasal layer of the diseased epidermis, having several granules (GR). The nucleus (N) is indented and the cytoplasm is partly retracted. The retraction of the cytoplasm may be a preparation artifact. (× 25,000)

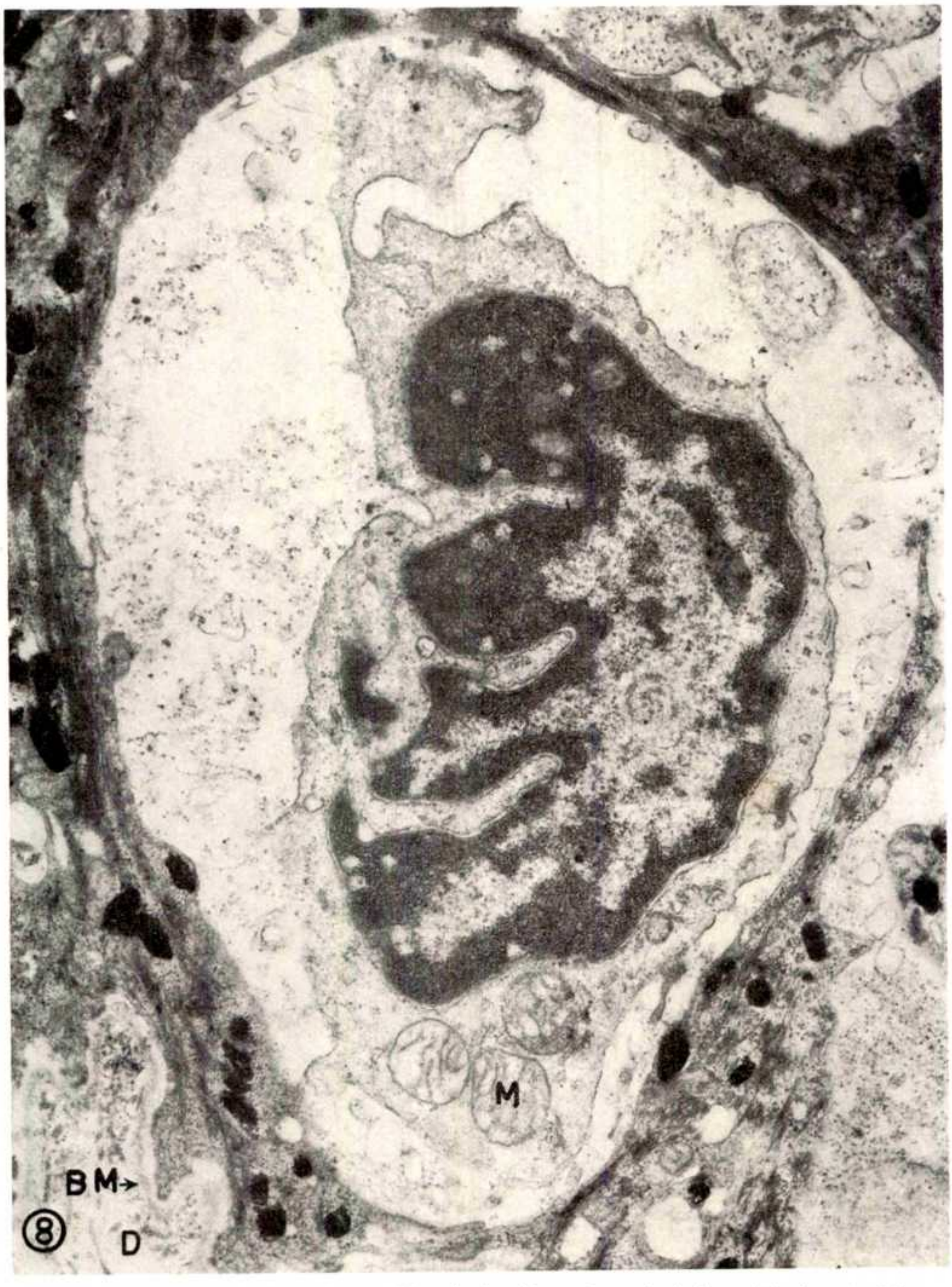

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FIG. 8.—A degenerating Langerhans cell in the basal layer from the lesion, with characteristic convoluted nucleus (N). The cell cytoplasm is mostly retracted and atrophic. A few mitochondria (M) are also present. The retracted cytoplasm can be a preparation artifact. (× 21,000)

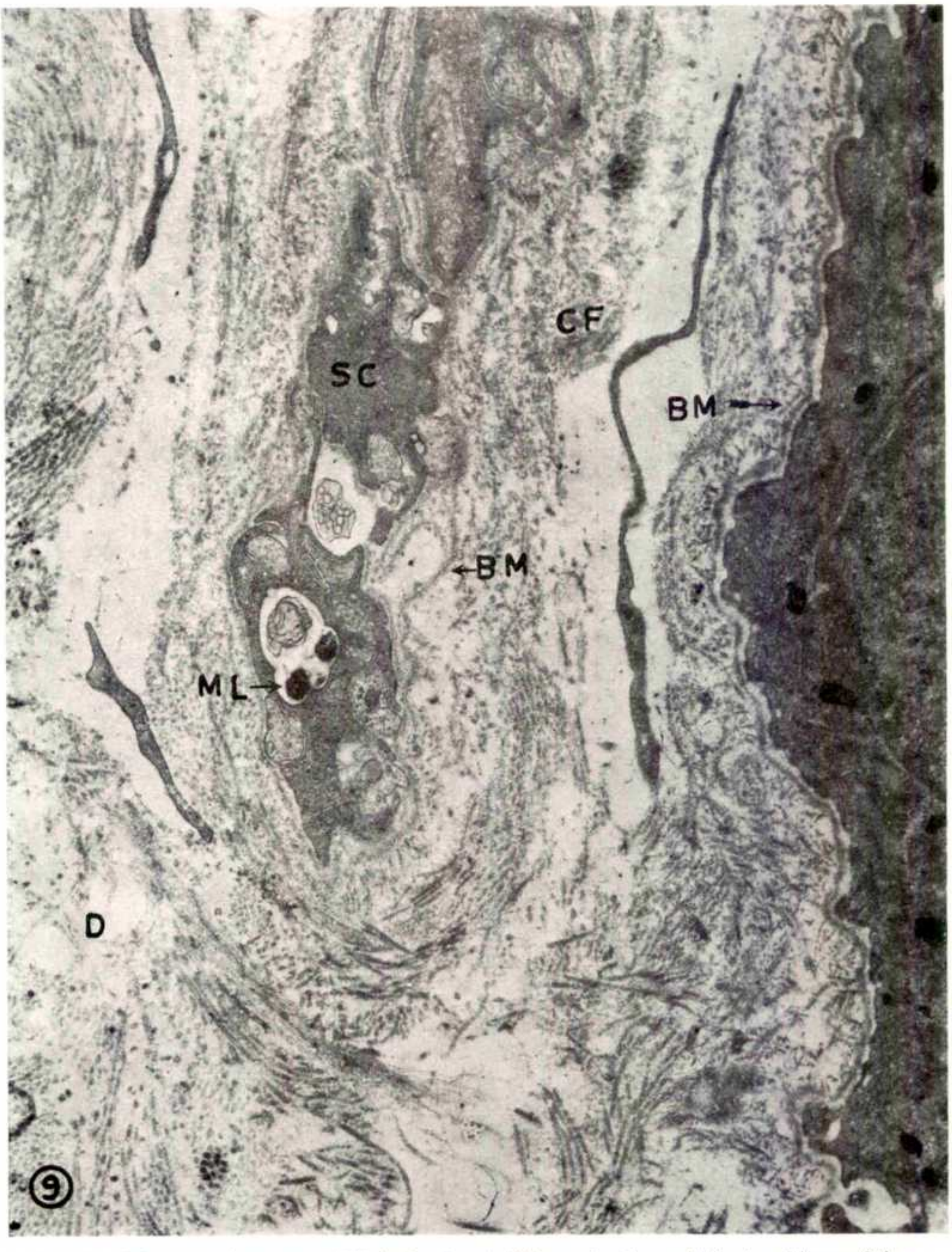

FIG. 9.—Schwann cell processes (SC) in the dermis (D) seen just beneath the basal layer of the epidermis. There is reduplication of the basement membrane (BM) of the Schwann cell. Note the cross sections of two *M. leprae* (ML). Many collagen fibrils (CF) surround the Schwann cell. (× 21,000)

DISCUSSION

In the present study of hypomelanotic leprous epidermis there was a marked reduction in the number of normal melanocytes. This confirms a previous study, where a significant reduction in the number of melanocytes in hypopigmented lesions of leprosy was reported (Nayar and Job, 1970). Further, there were many atrophic melanocytes showing signs of reduced activity and an increased number of active and atrophic Langerhans cells. Although not all these Langerhans cells contained the characteristic Langerhans granules, they had the typical indented or convoluted nuclei. Replacement of epidermal melanocytes by Langerhans cells has been reported previously in various hypomelanotic states. In vitiliginous epidermis normal basal melanocytes are totally replaced by Langerhans cells (Birbeck *et al.*, 1961). Breathnach *et al.* (1965) studied hypopigmented epidermis from the fore-arm of patients with piebaldism and reported a marked increase in Langerhans cells and a significant reduction in melanocytes.

Masson (1951) and Billingham and Medawar (1953) have hypothesized that Langerhans cells are " effete " or " worn out " melanocytes. However, it has been shown by electronmicroscopic studies that Langerhans cells are perfectly healthy (Breathnach, 1963) and are known to produce special granules and even melanosomes (Breathnach, 1964; Zelickson, 1965). Several healthy and active Langerhans cells were found in leprous epidermis, both superficially and in the basal layer of the epidermis. Further, a number of atrophic melanocytes with scanty inactive cytoplasm were also seen in the same specimen of epidermis. The theory of Masson that the Langerhans cell is an effete cell may not be acceptable, although melanocytes undergoing degenerative changes may possess indented nuclei.

Richter (1956), in a light microscope study, found that, in early cases of leprosy, Langerhans cells were greatly reduced in number and a majority of them were degenerated. Breathnach *et al.* (1962), in a later electronmicroscopic study of epidermal specimens from different types of leprosy, could not confirm this finding. In the 2 cases of indeterminate leprosy we studied there were atrophic changes in a number of Langerhans cells, although these changes could in part be explained as preparation artifacts. However, these cells were more numerous than in the control specimen and seemed to replace the melanocytes which were reduced in number.

Prabhakaran *et al.* (1971) have reported that *M. leprae* possesses an enzyme o-diphenol oxidase which oxidizes Dopa and have suggested that utilization of Dopa by *M. leprae* is the cause of hypopigmentation. The mere presence and multiplication of *M. leprae* may not be the reason for hypopigmentation, as the bacilliferous lepromatous lesions show the least change in pigmentation, and *M. leprae* are not ordinarily demonstrated using routine methods of examination in the hypopigmented lesions of indeterminate and tuberculoid leprosy. Further, *M. leprae* are also not demonstrated inside melanocytes in these patients.

It has been shown in this study that hypopigmentation of skin in early lesions in leprosy is due to atrophy and decreased activity of melanocytes, together with replacement of the normal melanocytes by Langerhans cells. The exact pathogenesis of decrease in the number of melanocytes and their inactivity is not yet clear.

This study was supported by a grant from the Leprosy Mission, London, a grant from the American Leprosy Missions, Inc., New York, and by U.S. Government grant SRS-IND-31-67 from SRS PL-480 Funds. We would like to express our appreciation to Mr K. George William for secretarial help; and to Mr R. Shanthakumar and Mr B. Joseph for technical help.

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THE ROLE OF THE CIRCULATION IN MEASUREMENTS OF SKIN CONDUCTIVITY

ANN WOOLLEY-HART

Summary.—Measurements of skin conductivity using silver disc and multiplint electrodes have been described. Comparisons with skin temperature measurements using thermistor probes have been made, and a parallel between the two parameters demonstrated. A connection between skin conductivity and blood flow was suggested, and confirmed by the effects on conductivity of exercise and the intradermal injection of adrenaline and histamine under the electrode site. The implications of this are discussed and a possible role of such measurements in the clinic is mentioned.

It is customary to consider the resistance of the skin to the passage of direct current as a function of the thickness of the stratum corneum and its moisture content (Tregear, 1966). Thus, the skin galvanic responses investigated by psychologists are said to have their origin in a temporary increase of permeability of the cells of the sweat glands. There is, however, the evidence from earlier German workers that epidermal cell permeability changes are involved (Gildermeister, 1912; Ebbecke, 1912), and Shaver *et al.* (1962) observed an epidermal component in their experiments to investigate the site of the skin potential response. In addition, Darrow (1927, 1929) reported observing maximal sweating with poor response amplitude and vice-versa, while Richter (1929) concluded one of his many papers with the comment that factors involved with temperature control appear to play a role.

Thus, despite the accepted view regarding thickness and moisture content of the stratum corneum, there are grounds for considering that some other factor might play a role. The most likely one to be involved here is the circulation with respect to either the diameter of, or rate of flow of blood, in the small vessels of the skin. This is because the body is a volume conductor and the bulk of resistance lies therefore in the skin immediately under the electrodes (Suckling, 1961). In this region, therefore, blood flow is the parameter most likely to be capable of undergoing sudden rapid changes such as are seen in the skin resistance response, etc. In support of this theory it is interesting that the areas of lowest resistance and maximum response are the fingertips; the palms, then the dorsum of the hand, and finally the forearm follow. Not only does the density of sweat glands follow this order but also the density of the arterio-venous shunts, which can be up to 250/mm^{-2} on the fingertips (Rothman, 1954). On the forearm there are probably none at all. These shunts are also found between arterioles and venules and at metarteriole level. The calibre of these smaller vessels is under both local metabolic and central nervous control.

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In the body an electric current is carried by ions, and the faster these can move, the higher the current, since by definition current is charge per unit time. Thus any factors aiding ion movement will increase the conductivity. Such factors are likely to be involved with rate of formation and removal of tissue fluid and with the permeability of the intervening cells. Tissue fluid formation is linked with blood flow, and it is also known with regard to the skin that the epidermis, capillaries and nerves form a functional unit, with changes in one component affecting another (Vickers, 1967). This is mediated via ion concentration changes.

This would mean then that, not only would local conditions affect the calibre and permeability of the smaller vessels, but conversely, the calibre and permeability of the smaller vessels, would affect the neighbouring structures, e.g. living epidermal cells and their permeability. An increase in permeability would aid ion transport and hence increase conductivity.

For these reasons it was decided to investigate the correlation between circulatory factors and the conductivity of the skin. Therefore the following simple manoeuvres were undertaken with the object of altering the circulation to the skin, either generally or locally, via reflex mechanisms or drug action.

1. Reflex vasodilation and constriction.
2. Reactive hyperaemia.
3. Intradermal injection of adrenaline and histamine.

If circulatory factors play a role in skin conductivity, drugs affecting the skin blood vessels might be expected to affect the conductivity also. Therefore, compared to a control, adrenaline should lower the current and histamine raise it.

4. The effects of exercises.

It was felt that if the rate of blood flow is important, it should be possible to alter this by deliberately manipulating the state of venous tone. Veins are the capacitance vessels, and an increase in tone increases the venous return and hence the cardiac output, thereby stimulating the entire circulation. Burch (1960) has demonstrated the exquisite sensitivity of veins to stimuli, ranging from coughing to unexpected noises. His results on change of tone in an isolated venous segment have a striking similarity to skin resistance responses. Since the fingertips are well supplied with an abundance of thin-walled, sub-papillary, venous plexuses, it does suggest that tone changes here could participate in the skin resistance responses, i.e. a temporary increase in venous tone acts as a "vis a tergo" and temporarily increases flow rate. A preliminary test had already shown that 100 % changes in fingertip current could be obtained from a subject lying on her back and raising her legs. For this reason, Yoga exercises were chosen, as they are slow, controlled movements aimed at stretching muscle groups and increasing blood flow rather than " toning up " and heat production.

MATERIALS AND METHOD

The actual technique employed for the measurement of skin conductivity is of considerable importance. If changes in the current values are being sought, two important criteria must be met. (1) The resistance of the supply circuit must be negligible in comparison with the resistance of the measuring circuit; (2) the voltage drop across the current meter must be negligible in comparison with the voltage applied to the subject. A circuit satisfying both the conditions was specially designed (Woolley-Hart, 1972) and consists essentially of a potential divider across a 9 V battery, with an electronic micro-ammeter to register current. In all experiments described in this paper the applied e.m.f. was 3 V (except for the exercises at 4·5 V).

The electrode system used can also affect the result, as can be seen from Fig. 1. In all cases the electrodes used had an area of 1 cm², and the site of measurement was the forearm. In A, the rise of current with time is due to an accumulation of moisture under a dry disc electrode, as already described by Tregear (1966). In B, multi-point electrodes are used, and these permit penetration of the stratum corneum (Lewes and Hill, 1967). The resistance of this layer is thereby reduced and the current is higher. This gives rise to a polarization e.m.f., and it is this which causes the high apparent resistance of the skin to direct current, for it effectively reduces the applied voltage. This aspect is fully discussed by David (1922), Gildermeister (1912) and Krauss *et al.* (1953). It arises from the lack of freedom of ionic movement in the tissue, which causes a build-up of ions. Lastly, in C, electrode jelly has been applied, and here the current is marginally higher than with the multipoint electrodes. After an initial fall due to polarization, there follows a rise in current. This can be attributed to ion build-up in the tissues reaching a value sufficient to alter the membrane properties and hence permeability of the underlying structures (Gildermeister, 1912).

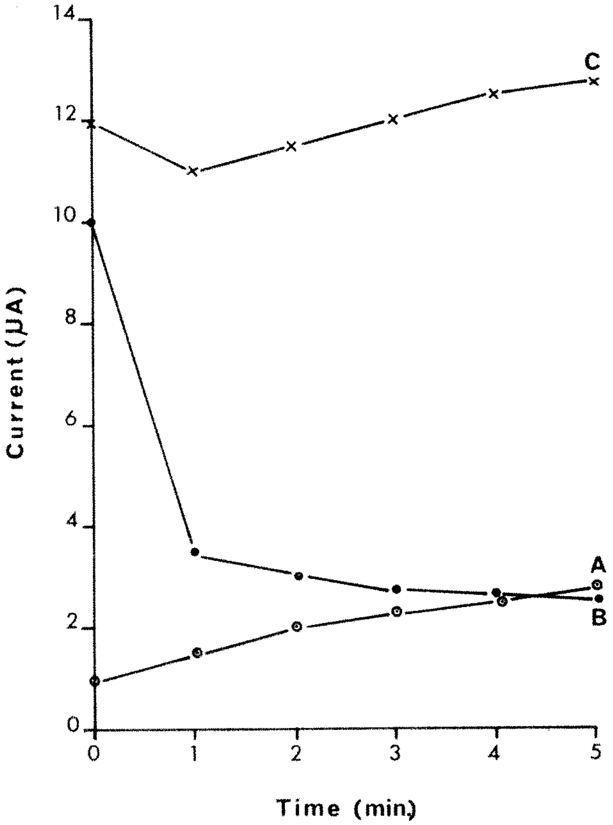

FIG. 1.—Effect of different electrode systems on current-time relationships. A = Dry disc electrodes; B = Multipoint electrodes; C = Electrode jelly + discs. Applied e.m.f. = 3 V.

In order to minimize these effects, all the experiments except one (Exercises) were conducted using dry electrodes which were placed on the chosen site only when a reading was taken. With the multipoint electrodes the highest value was recorded, and with the disc electrodes a reading after 10 seconds was taken.

The sites of measurement in all the experiments were either the pulp of the left second and third finger and/or the left forearm, ventral aspect, 1 cm above the distal crease of the wrist joint.

The multipoint electrodes were strapped with Sellotape on to the fingertips and then pressed firmly on the skin. At the wrist joint they were held firmly *in situ* with a "Cristalok" valcro tourniquet and pressed firmly. Prior experiment had shown this to give reproducible results. There was 1 cm distance between the electrodes. The electrodes were made of sheet silver 0·7 mm thick, with holes "stabbed" out. The disc electrodes were also of silver and of identical area (1 cm^2) to the others. They were sunk into a specially constructed plastic base, with a handle, and held by hand firmly on a previously marked area. With disc electrodes the role of pressure is minimal compared to multipoints. In fact, early work showed that identical results were obtained when disc electrodes were held on, compared with an elaborate technique involving inflating a small, specially constructed, sphygmomanometer cuff to 50 mm Hg pressure over the electrode site. The electrodes were not chlorided as, once again, at the low levels of current (up to 20 μA) used over short time intervals (10 seconds), there was no difference observed between them and non-polarizing electrodes.

In 3 of the experiments parallel readings of temperature were taken. Small thermistor probes were used (5 mm diameter) and attached to a circuit designed to give a read-out in T°C. The equipment was made by Light Laboratories of Brighton, Sussex and kindly lent by Mr Challoner of St John's Hospital for Diseases of the Skin. The probes were strapped to the skin with Sellotape and left *in situ* during the experiment. Readings were taken at the same time as those for current.

The different methods applicable to the individual experiments and, where necessary, the reason for doing it, are given below.

1. *Reflex Vaso-dilatation and Constriction*

Here the object was to change the blood flow in the arm under measurement by immersing the other in hot or cold water at 45°C and 5°C respectively. By the use of cold water it was hoped to reduce the role of sweat gland activity. Accordingly, temperature probes and dry disc electrodes were applied to finger and forearm sites and control readings taken before immersion in the hot or cold water. Further measurements were made at 10 min, 15 min and 25 min after immersion, following which the arm was removed and allowed to return to room temperature, with readings taken at 5-min intervals. A similar experiment was repeated, using multipoint electrodes, but the arm was removed from the hot water and put straight into the cold water.

2. *Reactive Hyperaemia*

In one experiment changes of electrical conductivity and temperature were monitored simultaneously before, during and after occlusion of the blood supply to the arm by means of a sphygmomanometer cuff. The sites were as before, and the cuff was inflated to 200 mm Hg for 7 min. In the second experiment comparisons of multipoint electrodes applied intermittently (to take the reading only) and continuously were made with the continuous application of disc electrodes. The readings were from the forearm only, and occlusion was for 3 min at 200 mm Hg.

3. *Intradermal Injection of Adrenaline and Histamine*

An injection of 0·05 ml of 1/10,000 adrenaline (or histamine) in normal saline was made intradermally under the previously-marked forearm electrode site. Care was taken

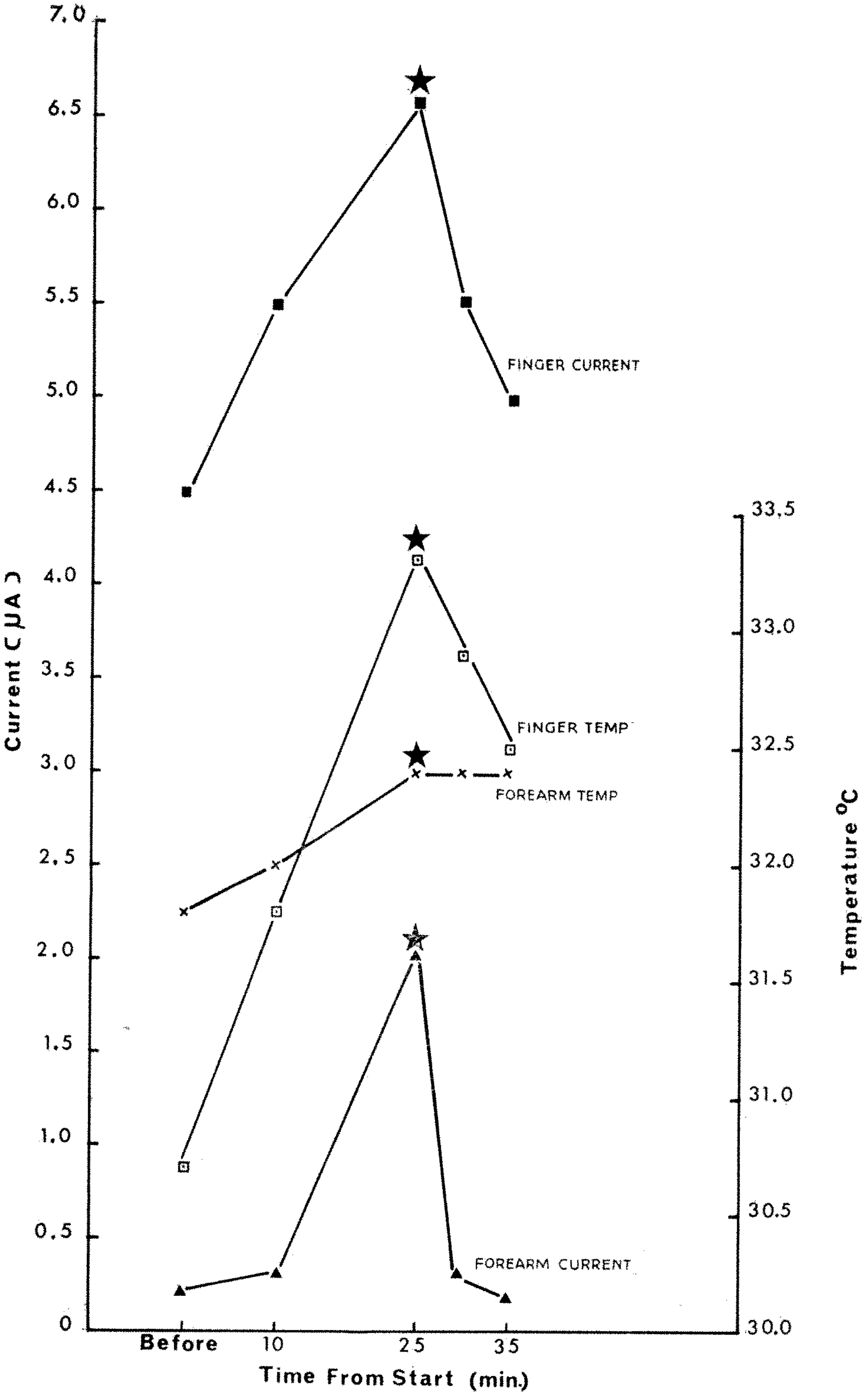

Fig. 2.—Changes in current and temperature on the left arm and fingers when the right arm is placed in hot water for 25 min. and then removed.
★ = Arm removed from hot water. Applied e.m.f. = 3 V Water temperature is 45°C. Disc electrodes.

that the point of needle entry lay outside the electrode area. Disc electrodes were used, a control reading taken at the beginning, and then readings taken every 5 min for 1·25 hours. Finally, as a control, an equal volume of saline was injected.

4. *The Effect of Exercises*

A programme of Yoga exercises, lasting 40 min, was monitored by the application of cm^2 disc electrodes strapped to the fingers with Sellotape. The electrodes remained *in situ* throughout. The exercises start with deep breathing and progress to slow, controlled movements. Essentially, changes of posture are involved from lying down to headstands. The pulse rate before and after exercise was taken.

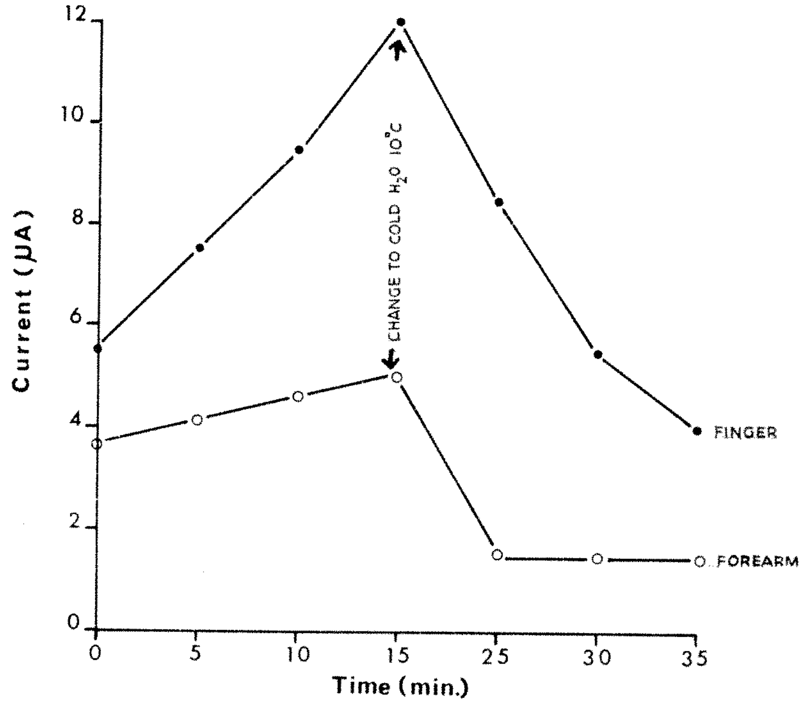

Fig. 3.—Current changes in finger and forearm when multipoint electrodes are applied to the left arm and the right is put first in hot, then in cold water. Applied e.m.f. = 3 V.

RESULTS

1. *Reflex Vasodilatation and Constriction*

The results of temperature and current measurements are shown in Fig. 2. In the fingertips there is a parallel change in both parameters, which is in keeping with the physiological role of the extremities in temperature regulation. Here blood flow is increased, but it is not customary to consider an increase in the sweat gland activity of the fingertips as resulting from changes in temperature, but more from emotional or psychological factors. The forearm readings show a modest rise in temperature accompanied by a tenfold increase in current after 10 min.

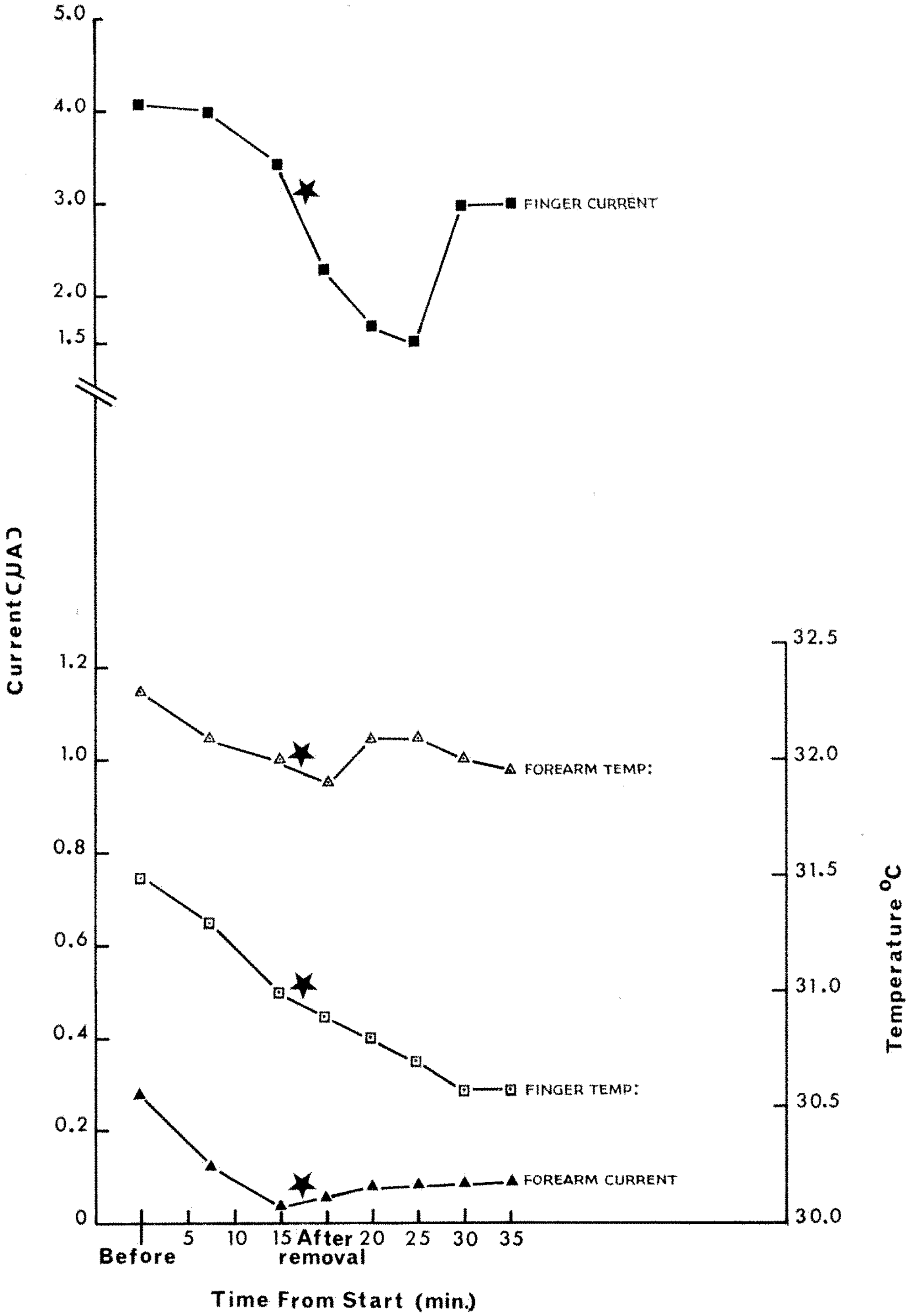

FIG. 4.—Current and temperature changes on the left forearm and fingers when the right arm is placed in cold water (5°C) for 15 min and then removed.
★ = Arm removed from cold water; Applied e.m.f. = 3 V. Disc electrodes.

This large increase must be attributed to sweating and consequent lowering of the resistance of the stratum corneum.

Fig. 3 shows the results of reflex vaso-dilatation on the 2 sites if multipoint electrodes are used. As stated, these electrodes penetrate through the stratum corneum and reduce the resistance of this layer, in this case by a factor of circa 20 : 1. A comparison of Fig. 2 with Fig. 3 shows the following points:

(1) The forearm current has a value more nearly that of the fingertip.
(2) The change in forearm current due to heating is very much less than when disc electrodes are used, as moisture effects are much reduced.
(3) The starting current values on the fingertip are not very different (1 μA). The rise on heating when using multipoint electrodes is double that with disc electrodes.

It would thus seem that, by reducing the dependency on moisture content, the multipoint electrodes show up events at deeper layers. These events are compatible with changes in blood flow and in the manner in which the two areas might respond.

The results of cooling are shown for multipoint electrodes in the second half of Fig. 3 and for disc electrodes in Fig. 4. The multipoint electrodes show a rapid fall-off of current in both sites, which continues on the fingers but reaches a steady state on the forearm. The disc electrodes show a parallel fall-off of both parameters on both sites. However, once the arm is removed from the cold water, differences appear. The subject by this time is cool and continues to cool down from remaining stationary in surroundings of 18°C, as the finger current and temperature continue falling, whereas the forearm readings show a slight rise and then level off. In both sites it is interesting to note that the current changes before temperature. This can be accounted for by the thermal capacity of the intervening tissues.

2. *Reactive Hyperaemia*

The results using temperature probes and disc electrodes are shown in Fig. 5. During occlusion there is a fall-off at both sites of both parameters. Again, the sites respond according to their physiological characteristics. On release of the cuff (at the star) the forearm current rises at once, and that of the fingers more slowly and never to the original value on account of cooling, as can be seen from the finger temperature curve. This also explains the subsequent fall-off in all parameters, as again the subject was stationary in a cool room.

Fig. 6 shows how the mode in which the different electrodes are used can alter the information obtained from temporary occlusion of the circulation. The readings are taken from the forearm only. Occlusion occurs between the stars. The use of intermittent multipoint electrodes (upper curve) demonstrates the effect of circulatory occlusion on the skin conductivity extremely well and also the hyperaemia that follows. This is completely masked by the continuous application of this electrode (lowest curve) owing to polarization events. The middle curve, using disc electrodes, shows how there is a reduction of insensible perspiration during occlusion. This is of interest, as it has some bearing on the source of the insensible perspiration which Bettley and Grice (1965) do not consider to be related to blood flow, although Newburgh and Johnston (1942) do.

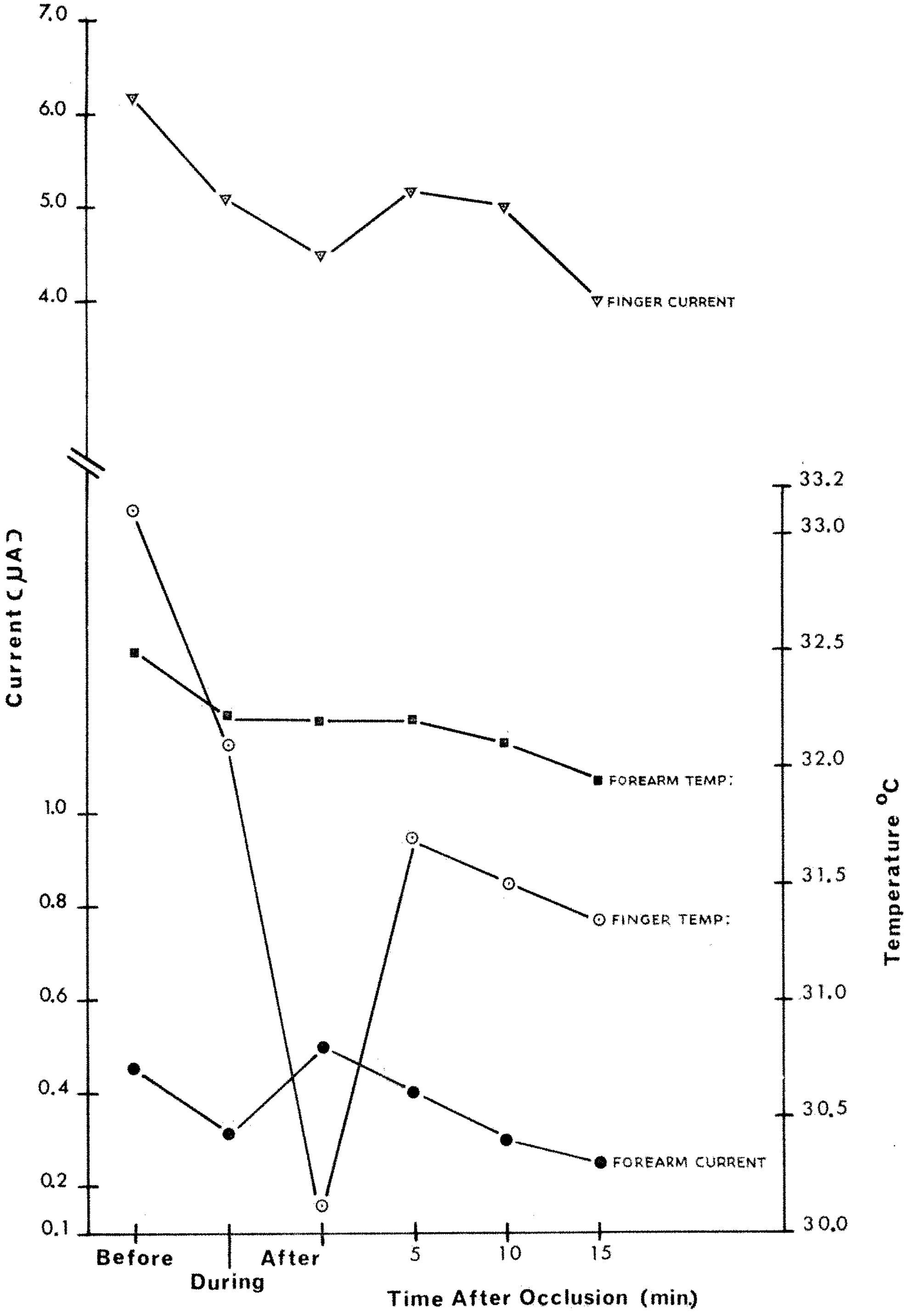

Fig. 5.—Effect of vascular occlusion on finger and forearm temperature and current. Cuff at 200 mm Hg for 7 min. Readings continued for 15 min after release. Disc electrodes. Applied e.m.f. = 3 V.

3. *Intradermal Injection of Adrenaline and Histamine*

The results are shown in Fig. 5. They were not what was expected, but can be easily explained by an examination of the control saline curve. An extra bulk of fluid has been introduced into the skin. This causes a slight increase in current, which has returned to the control value in 10 min. It is well known that radioactive sodium is cleared from the skin by a fast and slow component. The fast component is known to be circulatory, and the slow one has been considered as due to exchange with non-active diffusable sodium (Challoner, 1971). It could,

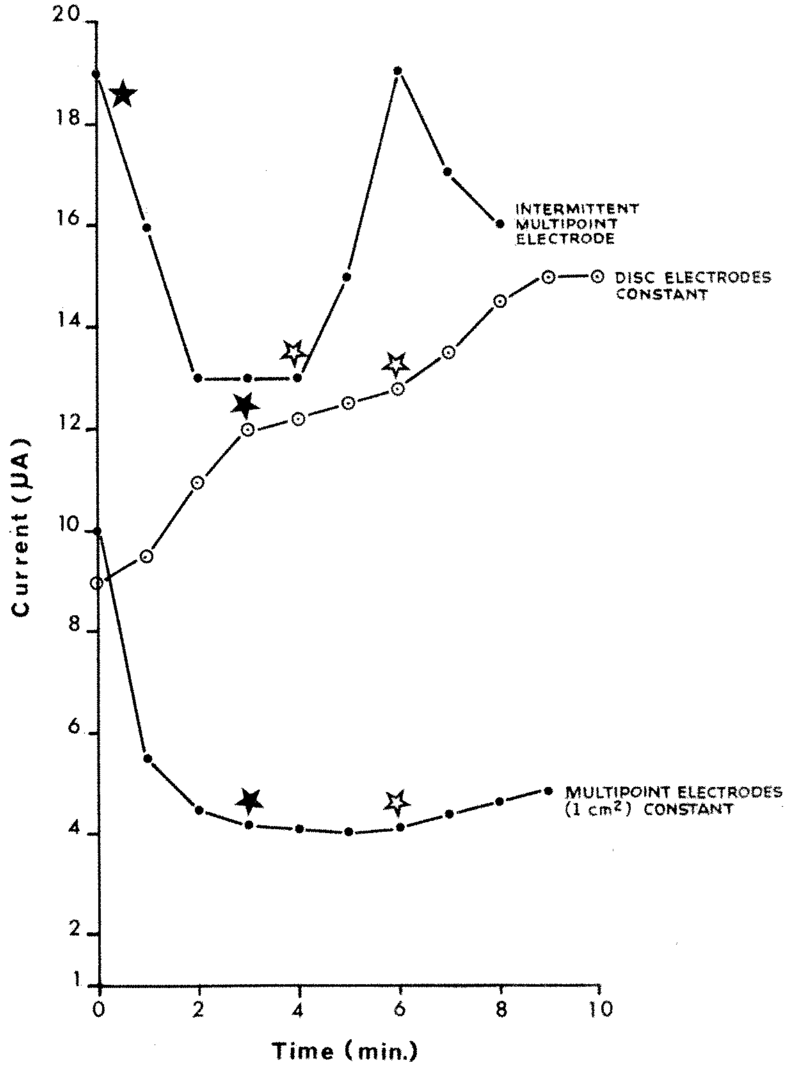

Fig. 6.—Effect on current when different electrode systems are used for measuring conductivity during occlusion. Cuff at 200 mm Hg for 3 min.
★ = inflation. ☆ = deflation.
Forearm readings. Applied e.m.f. = 3 V.

of course, also be due to diffusion through the intercellular spaces. Thus, after adrenaline, the fast component is absent and the bulk of the fluid remains in the tissues until the effect of the adrenaline wears off. Meanwhile, under the electrode site, there is more fluid to carry the ions, and the current is higher than the control. The histamine causes oedema and stasis from increased capillary permeability. Theoretically, this should lead to increased facility of ion movement, but there is also stagnation in the tissue spaces. This results in the current after the first 10 min being less than before the injection.

These results suggest that, not only is vessel calibre important, but also rate of flow. In support of this is the known higher resistance during sleep and meditation (Wallace, 1970) and hypnotic trance states (Cade and Woolley-Hart, 1971). During sleep, etc. the heart rate is reduced and there is vaso-dilatation, so the rate of flow is slower.

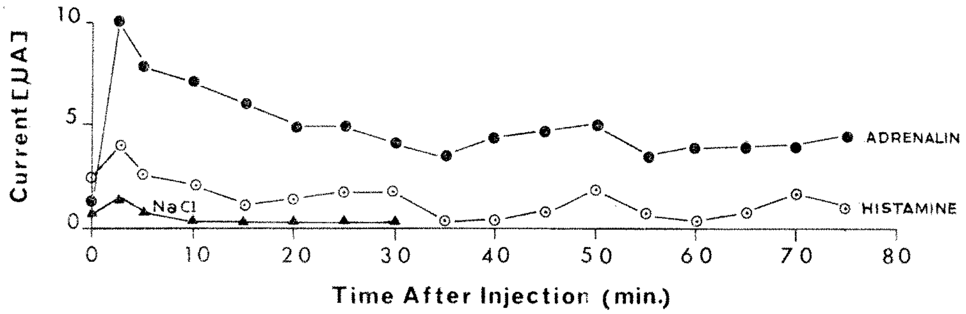

Fig. 7.—Effect of an intradermal injection of adrenaline, histamine and saline on current. Injection site under the electrodes. Applied e.m.f. = 3 V. Disc electrodes. ● = 0·05 ml 1/10,000 adrenaline in NaCl. ⊙ = 0·05 ml × 1/10,000 histamine in NaCl. ▲ = 9% NaCl.

4. *The Effect of Exercises*

The results of the observations are shown in Fig. 8. Each dot represents the current value as a change in position occurred. During the deep breathing, inspiration shows an increase in current, and expiration a fall-off, which does not return to starting value. There is therefore a gradual rise in current. The increased negative intra-thoracic pressure during inspiration aids venous return. During inspiration there is also increased activity from the vaso-motor centre due to radiation from the respiratory centre.

The exercises which follow are punctuated with periods of relaxation on the floor. During relaxation the current values are lowest. They rise as a movement is initiated, but the increase does not last during holding of the new position. The highest values were obtained in achieving those positions that put most strain on the abdominal muscles, thereby compressing the vena cava. The pulse rate rose from 54 to 60, which indicates a slight increase in the rate of blood flow over the period of exercise. This is concomitant with the gradual rise in current.

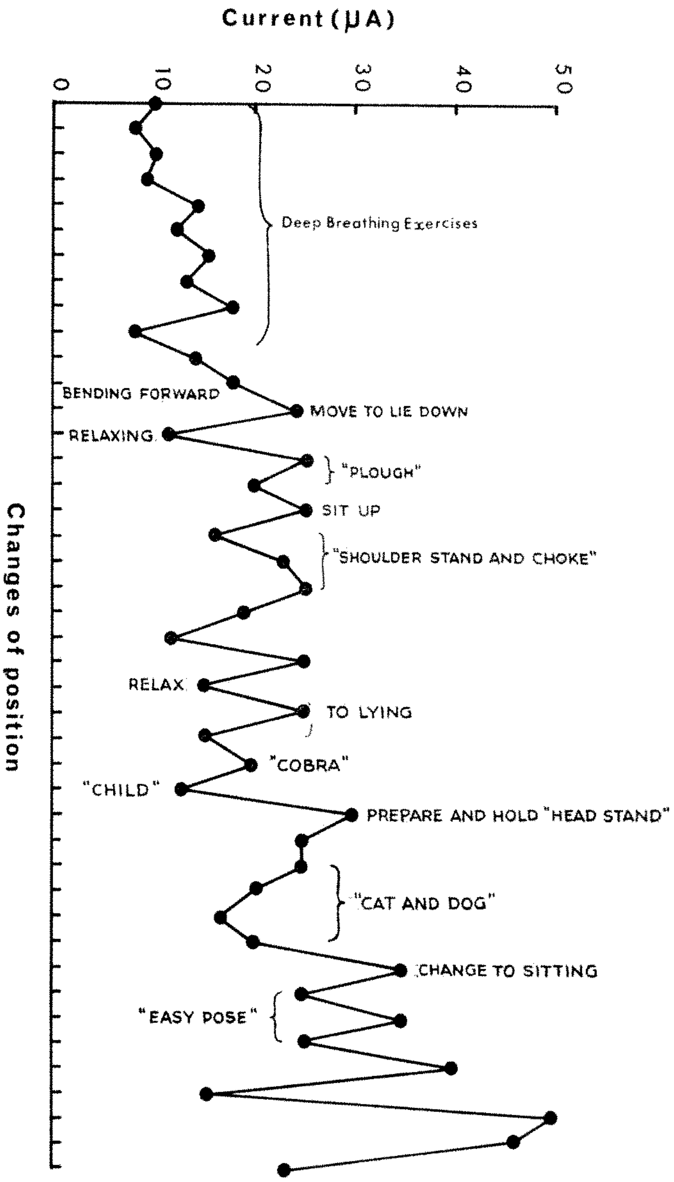

FIG. 8.—Effect of changes of position on current during a programme of Yoga exercises. Disc electrodes. Applied e.m.f. = 4·5 V.

DISCUSSION

The observations described implicate a circulatory factor in the passage of a current through the skin. This factor becomes apparent only when the masking effect of the high resistance of the statum corneum has been reduced, without recourse to electrode jelly.

It should be stressed that this approach is complementary to the more accepted one of sweat gland permeability levels or changes in activity. There are, therefore, two factors involved instead of one. The experiments described stress the importance of rate of blood flow as well as the calibre of the vessels. This means that, during vasodilatation, there must be an increase in heart rate or cardiac output to keep the flow rate the same. For example, during sleep there is vasodilatation and slowed heart rate, and there is low skin conductivity. This can be attributed to reduction in sympathetic tone which applies to sweat gland activity as well as vessel calibre. On the other hand, during exercise, the sympathetic tone is increased, but the overriding factor is the need for heat loss. There is therefore vasodilatation of the fingers and toes due to reduction in vasomotor tone, and in the other skin areas the release of bradykinin from sweat gland activity causes dilatation of the vessels. Obviously the conductivity of the skin is high here. In fact, Herxheimer (1960) has not observed forearm sweating without vasodilatation. He explains the cold palmar sweating of fear and nausea as being due to the massive vaso-constriction which masks the vaso-dilatation due to the release of plasma kinins.

In this context Burton and Taylor (1940) noted that psychogenic stimuli produced parallel changes in vaso-constrictor tone and sweat gland activity. Vasoconstriction will produce a momentary increase in flow rate. In spontaneous panic attacks Lader and Matthews (1970) have demonstrated that changes in skin conductivity are accompanied by parallel changes in heart rate, finger pulse volume forearm extensor muscle tone and forearm blood flow.

Thus one can see that sweating and skin blood vessel calibre are intimately linked, but the extent to which they run parallel will be determined by the physiological control mechanisms of overriding importance at that moment. In this respect the need for constant internal temperature will always take priority. The role of blood flow in skin conductivity measurements would also explain the observations of Darrow (1927, 1929) and the comments of Richter (1929), as mentioned in the introduction. It could also account for the epidermal component found by Shaver *et al.* (1962), for, from Vicker's (1967) approach, skin blood flow changes would cause changes in the permeability of the epidermal cells.

An understanding that skin conductivity measurements are not just associated with the thickness and moisture content of the stratum corneum or permeability of the sweat glands but also related to skin blood flow rate makes it a tool of more interest. For instance, one could use such measurements as a means of monitoring the effect of drugs on the circulation. In the psychological field it becomes a tool of great importance for monitoring the lability of the circulatory system to stimuli or problems of a threatening nature (Maxwell Cade and Woolley-Hart, 1971). It actually highlights the role of circulatory change in psychosomatic medicine, for mental "stress" can fix vasomotor tone in such a way that skin resistance responses are no longer found (Maxwell Cade, 1971). Regelsberger (1949) found that the normal daily fluctuations in skin conductivity were absent in subjects with anxiety states or catatonic schizophrenia. These patterns were also missing in localized areas of skin overlying a diseased viscus or generally absent in systemic diseases like Addison's disease of hyperthyroidism. Regelsberger (1949) found low conductivity in the former and high conductivity in the latter. In short, the question of skin conductivity measurements as an aid to examining the cutaneous projection of visceral disease by reflex pathways (as described by Hansen and Schliack, 1962) takes on new interest.

It is proposed to undertake further work to investigate the usefulness of skin conductivity measurements along the lines mentioned above.

I should like to thank the Polytechnic of Central London for a Research Fellowship which enabled me to undertake this work. In addition, I wish to express my thanks to Dr Jack Simmons for unfailing help and interest in all aspects of the work. I am also indebted to the technical staff of the Mathematics and Physics Department for their help in loaning or making equipment. Lastly, I should like to thank Dr Colin Ramsay, of St John's Hospital for Diseases of the Skin, for discussion and help given.

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CHANGES IN THE FATTY ACID COMPOSITION OF THE PHOSPHOLIPIDS, TRIGLYCERIDES AND FREE FATTY ACIDS WITH DEPTH IN THE COW SNOUT EPIDERMIS

V. J. W. LONG

Summary.—Lipids were extracted from slices taken horizontally through the epidermis of the cow snout. The fatty acid compositions of the phospholipids, triglycerides and free fatty acids of each slice were determined. There was a decrease in the proportion of octadecanoic acid in the phospholipids and triglycerides, and marked increases in the proportions of octadecenoic and octadecadienoic acids in the triglycerides towards the skin surface. There was little consistent change in the composition of the free fatty acids through the epidermis. Possible explanations for these findings are discussed.

STUDIES on the respiration of skin slices in the presence of various inhibitors have shown that fatty acid is an endogenous substrate of skin (Yardley and Godfrey, 1967). Further *in vitro* experiments showed that, when skin slices were cultured in medium without added glucose, the skin cells degraded endogenous phospholipid (Yardley and Godfrey, 1964; Long and Yardley, 1970). These results suggested that the skin cells can utilize fatty acids which are derived from the phospholipids. Furthermore, these *in vitro* experiments suggested that lack of glucose might induce skin cells to degrade endogenous phospholipid *in vivo* and therefore be important in the process of normal keratinization. The existence of a concentration gradient of glucose has been demonstrated in cow snout epidermis, and the phospholipid concentration may be correlated with the glucose concentration (Long, 1970). The glucose concentration falls steadily from the basal layer to low and relatively constant values in the upper layers of the epidermis. The phospholipid concentration increases at first; but, at the point at which the glucose concentration levels out, the phospholipid content starts to decrease and decreases markedly towards the skin surface. The concentrations of triglycerides and free fatty acids were small and constant in the lower layers of the epidermis, but they increased dramatically in the upper layers.

In a recent paper, Ansari *et al.* (1970) have compared the fatty acid compositions of the living layer and stratum corneum lipids of human sole epidermis. Human skin is not easily divided into more sections than the stratum corneum and living layer; consequently the possibility of contamination of the stratum corneum lipids by surface lipids cannot be ruled out. The cow snout, however, has a comparatively thick epidermis, from which 6 horizontal slices can be obtained. This enables the changes in the fatty acid composition of the lipids with depth in the epidermis to be determined. In this paper the changes in the fatty acid composition of the phospholipids, triglycerides and free fatty acids have been investigated.

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MATERIALS AND METHODS

Extraction of Lipids from Skin Slices

Cow snouts were obtained immediately after slaughter, washed with water, and the skin surface wiped lightly with an ethanol-impregnated swab. Six horizontal slices of epidermis (0·2 mm) were obtained consecutively, using a Castroviejo keratotome (John Weiss and Son, Wigmore Street, London, W.1) from a fixed area of the snout which was free of sebaceous glands. Portions of the slices were fixed in buffered formol-saline and stained with H. and E. The remainder of the slices were weighed and ground with chloroform-methanol (2 : 1, v/v) in a glass tissue grinder. Grinding and extraction were repeated twice, and the extracts of lipids taken to dryness under nitrogen. The residues were re-dissolved immediately in chloroform-methanol (2 : 1, v/v) and taken to dryness, again under nitrogen. This procedure was repeated once more to ensure cleavage of proteo-lipids (Ansell and Hawthorne, 1964). The residues were re-dissolved in chloroform-methanol (2 : 1, v/v) and the extracts of lipids washed by the method of Folch *et al.* (1957). The washed extracts were dried over anhydrous sodium sulphate.

TABLE I.—*Fatty Acid Composition of the Phospholipids from the Epidermal Slices Expressed as a Percentage of the Total Phospholipid Fatty Acids**

Fatty acid		Skin slice					
Carbon number	Probable identity	1 Stratum corneum	2	3	4	5	6 Basal layer
12·95	13 : 0	T	0·5	T	0·6	0·8	T
		—	—	—	—	—	—
14·00	14 : 0	3·6	5·2	4·3	4·3	3·1	1·4
		5·0	6·4	5·1	4·0	2·9	2·6
16·00	16 : 0	29·1	23·8	25·5	22·7	26·1	21·7
		40·6	29·4	30·2	27·6	26·0	30·1
16·38	16 : 1	8·0	10·2	8·4	6·6	6·0	4·5
		4·2	6·6	7·1	6·2	5·4	3·9
18·00	18 : 0	10·6	9·2	10·3	11·4	16·3	16·5
		10·3	8·2	8·9	10·5	12·2	15·3
18·32	18 : 1	20·0	26·7	27·2	27·4	27·4	24·6
		14·7	28·6	28·0	29·2	27·5	22·1
18·89	18 : 2	10·0	11·7	12·0	12·9	14·8	12·6
		5·8	11·3	11·8	13·2	15·4	14·4
19·40	—	—	—	—	—	—	—
		2·4	0·1	0·3	0·3	0·2	0·1
19·65	18 : 3	0·7	0·8	0·8	1·0	1·2	1·1
		1·1	2·2	2·4	2·3	2·5	2·3
20·00	20 : 0	4·5	2·4	1·8	1·5	1·6	2·3
		4·7	1·4	1·0	0·9	0·9	1·3
20·38		1·8	2·3	1·5	1·2	1·4	1·4
		3·4	1·4	1·2	1·0	0·8	0·8
21·05	21 : 0	3·6	1·0	0·7	1·8	T	T
		—	—	—	—	—	—
21·26		1·3	1·5	1·8	2·4	0·5	3·2
		1·5	1·0	1·0	1·1	1·5	1·3
21·50	20 : 4	2·3	2·0	3·2	4·8	0·8	9·4
		1·2	1·3	1·4	2·1	3·0	3·8
22·00	22 : 0	4·5	2·7	2·5	1·4	—	1·3
		5·1	2·1	1·6	1·6	1·7	2·0

* The table shows the results of 2 experiments on different animals.

: 0; saturated fatty acid.

: 1, : 2, : 3, : 4; unsaturated fatty acids with 1, 2, 3 and 4 double bonds respectively.

T; trace.

—; not detected.

Fractionation of Lipids into Lipid Classes

Portions of total lipid extracts were applied under nitrogen to a thin layer (0·5 mm) plate (20 × 20 cm) of silica gel G (Merck A.G., Darmstadt, Germany) which had been activated at 110°C for 30 min. The plate was developed with light petroleum (boiling point range: 30°–40°C)—diethyl ether—glacial acetic acid (80 : 20 : 1, v/v/v), dried under nitrogen and sprayed with an aqueous solution of rhodamine 6 G (0·01%). The spots were visualized quickly with an u.v. lamp.

Preparation of Methyl Esters of the Fatty Acids from the Lipid Classes

The areas of silica gel containing the triglycerides, free fatty acid and phospholipid spots were scraped immediately after spraying the plate into glass-stoppered tubes containing methanol : sulphuric acid (95 : 5, v/v; 3 ml). The tubes were gassed with nitrogen and heated at 70°C for 2 hours. Distilled water (1 ml) was added and the methyl esters of the fatty acids were extracted by shaking 3 times with redistilled hexane

TABLE II.—*Fatty Acid Composition of the Triglycerides from the Epidermal Slices Expressed as a Percentage of the Total Triglyceride Fatty Acids**

Fatty acid		Skin slice					
Carbon number	Probable identity	1 Stratum corneum	2	3	4	5	6 Basal layer
14·00	14 : 0	4·5	4·2	4·3	5·2	2·5	3·1
		4·1	4·1	4·8	5·2	5·0	4·2
16·00	16 : 0	15·3	17·4	16·9	19·7	17·2	18·8
		16·4	16·6	23·3	24·6	25·1	22·2
16·41	16 : 1	11·2	8·5	6·8	4·9	5·3	6·1
		9·3	9·6	14·1	9·4	11·0	10·4
18·00	18 : 0	10·4	8·8	9·2	12·2	11·7	12·4
		7·7	7·1	7·9	10·0	12·2	13·8
18·33	18 : 1	33·7	32·8	21·0	16·1	17·5	20·8
		39·6	34·9	25·8	25·7	24·8	29·7
18·70		T	T	T	11·5	9·9	5·4
		T	T	T	T	T	T
18·91	18 : 2	13·4	12·1	5·0	6·2	5·8	6·7
		15·3	13·8	5·5	8·9	7·7	8·8
19·26		T	0·5	1·9	0·9	6·2	2·5
		T	2·3	4·0	5·8	7·7	4·7
19·65	18 : 3	1·0	1·0	1·5	2·3	5·0	2·3
		3·8	3·9	2·6	2·2	2·6	2·3
20·00	20 : 0	2·3	2·3	1·6	T	T	0·8
		0·9	1·5	1·5	0·9	0·8	1·2
20·35		5·0	4·9	2·0	2·9	3·0	3·5
		2·8	4·1	3·8	2·1	3·1	2·7
20·68		T	0·7	1·9	T	T	T
		—	—	—	—	—	—
21·06	21 : 0	3·3	3·3	16·2	13·1	13·6	7·9
		T	2·1	6·7	5·2	—	—
21·54	20 : 4	T	T	11·7	5·0	2·3	3·4
		—	—	—	—	—	—
22·00	22 : 0	T	1·0	T	T	T	T
		—	—	—	—	—	—
22·32		T	2·5	T	—	T	6·3
		—	—	—	—	—	—

* The table shows the results of 2 experiments on different animals.
: 0; saturated fatty acid.
: 1, : 2, : 3, : 4; unsaturated fatty acids with 1, 2, 3 and 4 double bonds respectively.
T; trace.
—; not detected.

TABLE III.—*Fatty Acid Composition of the Free Fatty Acids from the Epidermal Slices Expressed as a Percentage of the Total Free Fatty Acids**

Fatty acid		Skin slice					
Carbon number	Probable identity	1 Stratum corneum	2	3	4	5	6 Basal layer
14·01	14 : 0	2·7	3·7	5·6	5·0	2·8	3·0
		1·9	2·0	T	T	T	T
16·00	16 : 0	23·9	24·1	31·0	24·0	21·2	25·9
		23·9	23·5	12·6	21·9	16·9	23·7
16·34	16 : 1	4·4	5·3	6·7	9·2	4·5	5·0
		4·1	5·0	4·8	4·7	7·5	5·0
18·00	18 : 0	24·1	20·7	21·1	16·0	17·9	22·2
		16·8	17·4	10·5	16·9	13·0	19·3
18·32	18 : 1	16·6	13·8	11·2	9·3	18·5	13·6
		27·4	26·0	21·1	18·5	16·5	23·2
18·90	18 : 2	2·6	1·7	1·0	1·6	20·4	14·0
		1·1	2·9	1·0	2·1	1·9	3·1
19·35		0·9	1·4	2·3	4·5	0·8	2·2
		1·9	2·4	12·5	4·2	4·3	4·4
19·67	18 : 3	0·6	0·7	0·9	1·3	2·2	1·8
		0·5	0·9	T	T	T	T
20·00	20 : 0	1·3	4·9	4·2	2·1	1·0	2·7
		7·4	6·3	2·3	3·9	2·4	5·7
20·35		3·8	5·6	T	3·6	1·1	T
		5·4	7·2	13·1	4·4	7·5	7·2
21·03	21 : 0	6·8	8·0	14·4	15·7	6·4	9·6
		1·5	6·4	8·0	11·6	10·9	8·4
21·37		T	T	T	5·0	3·2	T
		T	—	10·7	9·0	19·1	T
22·00	22 : 0	12·3	10·1	1·6	T	T	T
		8·1	—	3·4	2·8	T	T
22·40		T	T	T	2·7	T	T
		—	—	—	—	—	—

* The table shows the results of 2 experiments on different animals.
: 0; saturated fatty acid.
: 1, : 2, : 3, : 4; unsaturated fatty acids with 1, 2, 3 and 4 double bonds respectively.
T; trace.
—; not detected.

(3 ml). The total hexane extracts were washed with water and dried over anhydrous sodium sulphate. The hexane extracts from the free fatty acids were evaporated with nitrogen and taken up in redistilled diethyl ether (0·3 ml). A few drops of a freshly prepared solution of diazomethane in diethyl ether were added to each sample until the yellow colour persisted. The ether and excess diazomethane were removed by heating to 60°C under a stream of nitrogen. The methyl esters of the fatty acids were dissolved in redistilled hexane. A 2-stage esterification process was used for the free fatty acids to ensure complete conversion to the methyl esters.

Gas-Liquid Chromatography of Methyl Esters of Fatty Acids

The methyl esters of the fatty acids were analysed using a Pye " series 104 " instrument equipped with a flame ionization detector and a 2 m column packed with 10% polyethylene glycol adipate, working at a temperature of 185°C. Methyl esters of myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidic acid, behenic acid and arachidonic acid (all supplied by Sigma, London) were used as standards. The fatty acids were identified by their retention time and quantitated by multiplying the height of each peak by the width at half the height.

RESULTS

Sections of the skin slices which were stained with H. and E. gave very similar pictures to those obtained previously (Long, 1970).

The fatty acid compositions of the phospholipids, triglycerides and free fatty acids from the skin slices are shown in Tables I–III. The fatty acid compositions of the lipids of the uppermost skin slice of the epidermis may be anomalous, due to contamination by surface lipids, oxidation of the lipids or to artefacts introduced

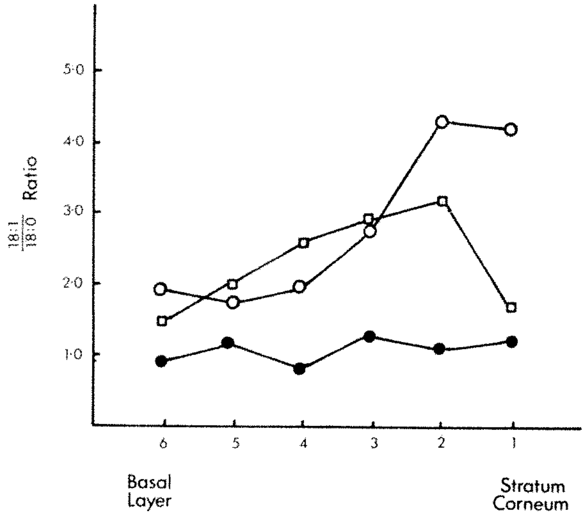

FIG. 1.—The changes in the octadecanoic/octadecanoic acid ratios for the fatty acid compositions of the phospholipids, triglycerides and free fatty acids with depth in the epidermis. The values plotted are mean values derived from the 2 experiments in Tables I–III.

—□—, phospholipids;
—○—, triglycerides;
—●—, free fatty acids.

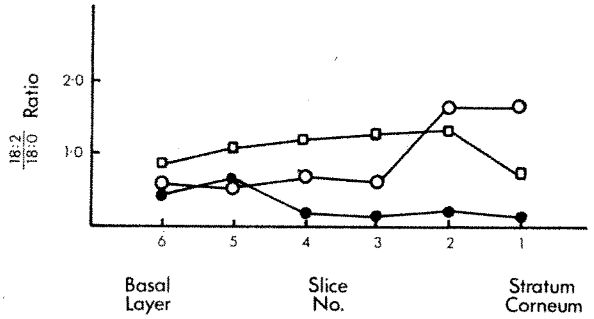

FIG. 2.—The figure shows the changes in the octadecadienoic/octadecanoic acid ratios for the fatty acid compositions of the phospholipids, triglycerides and free fatty acids with depth in the epidermis. The values plotted are mean values derived from the two experiments in Tables I–III.

—□—, phospholipids;
—○—, triglycerides;
—●—, free fatty acids.

by wiping the skin surface with an ethanol-impregnated swab before cutting the slices.

Although there are marked differences in the proportions of some of the individual fatty acids in the lipids of the 2 animals, with the exception of the surface layer, the fatty acid compositions of the phospholipids and triglycerides show consistent changes in pattern through the epidermis. This is seen most clearly by plotting ratios of the fatty acids in which changes are apparent. In this way any errors introduced in the fatty acid compositions of the lipid classes by the inclusion of peaks of uncertain identity can be avoided.

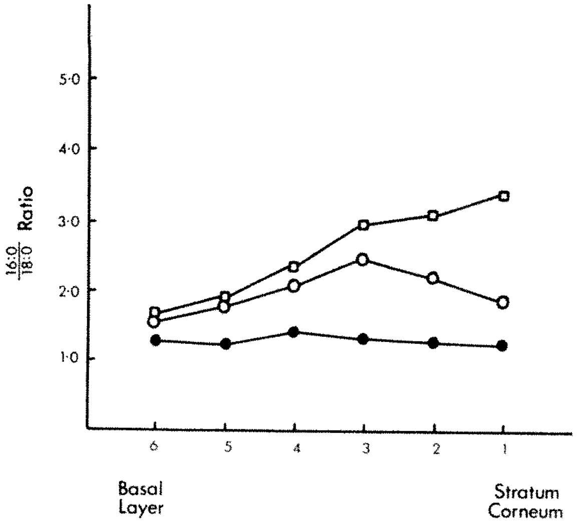

FIG. 3.—The figure shows the changes in the hexadecanoic/octadecanoic acid ratios for the fatty acid compositions of the phospholipids, triglycerides and free fatty acids with depth in the epidermis. The values plotted are mean values derived from the two experiments in Tables I–III.

—□—, phospholipids;
—○—, triglycerides;
—●—, free fatty acids.

The 18 : 1/18 : 0 ratios for the fatty acids of the phospholipids and triglycerides show marked increases towards the skin surface (Fig. 1). The 18 : 2/18 : 0 ratio for the fatty acids of the triglycerides also shows a marked increase towards the skin surface, but the increase in the 18 : 2/18 : 0 ratio for the fatty acids of the phospholipids is only slight (Fig. 2). The 16 : 0/18 : 0 ratio for the fatty acids of the phospholipids increases steadily towards the skin surface (Fig. 3).

In the case of the phospholipids, these changes are due chiefly to decreases in the octadecanoic acid content, rather than to increases in the proportions of octadecenoic, octadecadienoic or hexadecanoic acids. In the case of the triglycerides, the increases are due to marked increases in the proportions of octadecenoic and octadecadienoic acids towards the skin surface as well as to decreases in the proportion of octadecanoic acid.

There is little consistent change in the 18 : 1/18 : 0, 18 : 2/18 : 0 and 16 : 0/18 : 0 ratios for the free fatty acids through the epidermis (Figs. 1–3), although there are variations in the proportions of these fatty acids (Table III).

DISCUSSION

The differences in the fatty acid compositions of the lipids of the 2 animals are probably due to variations in the diet. However, the most striking finding is the marked increase in unsaturation in the fatty acid compositions of the triglycerides as the skin surface is approached. Not only is there an increase in the proportion of unsaturated fatty acids, but there is also an increase in the total amount of unsaturated fatty acids, since the previous work (Long, 1970) showed that the triglyceride concentration increased towards the skin surface. A possible explanation for this increase was suggested (Long, 1970). The phospholipids are degraded by phospholipases with loss of their fatty acids to glycerol phosphate which may be converted via phosphatidic acid into diglycerides and finally triglycerides. The increase in the triglycerides may be due to an increase in phosphatidate phosphohydrolase activity caused by lack of glucose. Presumably the activity of the diglyceride acyltransferase is increased at the expense of the enzyme converting diglyceride into phospholipid.

There is a decrease in the proportion of octadecanoic acid in the phospholipids towards the skin surface and also a decrease in the amount of octadecanoic acid, since the phospholipid concentration decreases towards the skin surface (Long, 1970). This may be evidence for a selective utilization of the fatty acids from the phospholipids by the skin cells. Ansari *et al.* (1970) have determined the total fatty acid composition of the living layer and stratum corneum lipids of human sole epidermis. These workers found a relative decrease in the palmitic and stearic acid composition of the stratum corneum compared with that of the living layer and suggested that oxidation of palmitate and stearate could supply the necessary energy for the late stages of keratinization.

In contrast to the changes observed for the fatty acids of the phospholipids and triglycerides, there was little consistent change in the free fatty acids through the epidermis.

The increase in the unsaturated fatty acid composition of the triglycerides may be due to a combination of the effect of temperature and oxygen concentration. The upper layers of the epidermis are presumably cooler than the lower layers, and there may be a significant contribution by atmospheric oxygen to the oxygen concentration in the tissue. Both these factors would favour an increased synthesis of unsaturated fatty acids. However, the increase in unsaturation of the fatty acids is observed only in the triglycerides and not in the phospholipids and free fatty acids. This is not surprising, since the phospholipids are degraded in the upper layers of the epidermis by phospholipases (Long and Yardley, 1972), whereas the increase in the triglyceride concentration is presumably caused by an increase in net synthesis.

The effect of temperature on the fatty acid composition of total lipids, or lipid classes, or in some cases individual phospholipids or fatty acids in goldfish (Knipprath and Mead, 1968), mosquito fish (Knipprath and Mead, 1966), arctic mice (Eybel and Simon, 1970), *E. coli* (Haest *et al.*, 1969), bacilli (Fulco, 1970), *Chlorella* (Patterson, 1970), seeds (Harris and James, 1969a) and plants (Harris and James, 1969b) has been investigated. Although there are significant species differences, there is invariably an increase in unsaturated fatty acids with decreasing temperatures. An increased uptake of ^{14}C-acetate into the unsaturated fatty acids with decreasing temperature has been demonstrated in goldfish (Knipprath and Mead, 1968), seeds (Harris and James, 1969a), and in bulb tissue (Harris and James, 1969b), and the effect of temperature on the mechanism of desaturation of palmitate has been studied in bacilli (Fulco, 1970).

The effect of oxygen concentration on fatty acid biosynthesis has been demonstrated in bulb tissue (Harris and James, 1969b) and seeds (Harris and James, 1969a). In the bulb tissue, the oxygen concentration was found to be limiting for the synthesis of unsaturated fatty acids at temperatures down to 10°C. An increase in oxygen concentration at a fixed temperature led to an increase in the formation of unsaturated fatty acids, and the elevated content of unsaturated fatty acids at lower temperatures in plants was attributed to an increase in the oxygen concentration in solution.

It remains to be demonstrated whether changes in temperature and oxygen concentration produce similar effects on fatty acid biosynthesis in skin.

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ALLERGIC CONTACT DERMATITIS FROM SESQUITERPENOIDS OF PLANTS

ADDITIONAL ALLERGENIC SESQUITERPENE LACTONES AND IMMUNOLOGICAL SPECIFICITY OF COMPOSITAE, LIVERWORTS AND LICHENS

J. C. MITCHELL, G. DUPUIS AND T. A. GEISSMAN

Summary.—By patch testing cross-sensitivity was not observed between the sesquiterpene lactone, alantolactone (from *Inula*) and ilicic acid, which is an unsaturated sesquiterpenoid lacking a lactone ring (from *Ambrosia*) and α-methylene-γ-butyrolactone (from *Tulipa*) which is the simplest structure containing the grouping that is characteristic of the active compounds; and protolichesterinic acid (from the lichen *Cetraria*) which is an unsaturated lactonic acid.

Some 50 additional potentially allergenic sesquiterpene lactones derived from plants are listed from the families, Compositae, Lauraceae, and Magnoliaceae; some lactones from the family Umbelliferae are also listed.

Previously we reviewed some 210 sesquiterpenoid compounds derived from Compositae species of plants and listed compounds which possessed a $=CH_2$ group attached to the lactone as allergenic or potentially allergenic (Mitchell and Dupuis, 1971). We have extracted from recent phytochemical work some 50 additional lactones and present these as an addendum to the previous paper, thus bringing the available information up to date in the dermatological literature (Table I). There are only a few compounds *lacking* the unsaturated lactones, and they are mainly found, except in the family Umbelliferae, accompanying compounds in plants *containing* the unsaturated lactones. A few compounds which have been previously reported are again listed, with additional plant sources. Potentially allergenic sesquiterpene lactones are present in plant species of the families Lauraceae and Magnoliaceae in addition to the family Compositae of the flowering plants, and also in the family Jubilaceae, genus *Frullania* (liverworts). In most cases, the lactones found in the Lauraceae and the Magnoliaceae are the same compounds as are found in Compositae, but they are not widely distributed, so far as is presently known, in these 2 families. So far, only sesquiterpene lactones which lack an unsaturated methylene group and which, therefore, are unlikely to be allergenic, have been found in the family Umbelliferae.

Table I lists 48 potentially allergenic sesquiterpene lactones which have not to our knowledge yet been tested for allergenic activity, but the great majority occur in genera of the Compositae family (notably *Artemisia*, *Ambrosia* and *Chrysanthemum*), which contain well-known sensitizing plant species, and all the lactones possess the previously described reactive $=CH_2$ group attached to the lactones.

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TABLE I.—*Potential and Reported Allergenicity of Sesquiterpenoids and Compositae Species*

			Immunological activity			
			Chemical compound			
Chemical compound	Botanical source	Tribe	Potential*	Reported	Genus	Species
Class Eudesmanolide						
Arbusculin-C	*Artemisia tridentata* Rydb.	Anthemideae	+	0	+	0
Arbusculin-C, D and E	*Artemisia arbuscula* Nutt. ssp. *arbuscula*	Anthemideae	+	0	+	0
Chrysanin	*Chrysanthemum cinerariaefolium* visiani	Anthemideae	+	0	+	0
Cumambrin-B oxide	*Artemisia tripartita* Rydb. ssp. *rupicola* Beetle	Anthemideae	+	0	+	0
β-Cyclopyrethrosin	*Chrysanthemum cinerariaefolium* visiani	Anthemideae	+	0	+	0
Dentatin-A	*Artemisia tridentata* Nutt. ssp. *tridentata*	Anthemideae	+	0	+	0
Dihydro-β-cyclopyrethrosin	*Chrysanthemum cinerariaefolium* visiani	Anthemideae	—	0	+	0
Farinosin	*Encelia farinosa* Gray	Heliantheae	—	0	+	0
Hybrifarin	*Encelia farinosa* Gray × *E. californica* Nutt. (Hybrid)	Heliantheae	—	0	+	0
Ludalbin	*Artemisia ludoviciana* Nutt. ssp. *albula*	Anthemideae	+	0	+	+
Ludartin	*A. carruthii* Wood ex. Carruth.	Anthemideae	+	0	+	0
Parishin-A, B and C	*A. tridentata* Nutt. ssp. *tridentata* f. *parishii*	Anthemideae	+	0	+	0
Ridentin-B	*A. tripartita* Rydb. ssp. *rupicola* Beetle	Anthemideae	+	0	+	0
Rothin-A and B	*A. rothrockii* Gray	Anthemideae	+	0	+	0
Rothin B	*A. tridentata* Rydb.	Anthemideae	+	0	+	0
Rupicolin-A and B	*A. tripartita* Rydb. ssp. *rupicola* Beetle	Anthemideae	+	0	+	0
Rupin-A and B	*A. tripartita* Rydb. ssp. *rupicola* Beetle	Anthemideae	+	0	+	0
Class Germacranolide						
Albicolide	*Jurinea albicaulis* Bge. var. Kilae	Cynareae	+	0	0	0
Calaxin	*Artemisia verlotorum* Lamotte	Anthemideae	+	0	+	0
	Calea axillaris DC.	Heliantheae			0	0
Chamisellin	*Ambrosia chamissonis* (Gray) Payne	Heliantheae	+	0	+	0

TABLE I—(cont.)

			Immunological activity			
			Chemical compound			
Chemical compound	Botanical source	Tribe	Potential*	Reported	Genus	Species
Chrysanolide	*Chrysanthemum cinerariaefolium* visiani	Anthemideae	+	0	+	0
Ciliarin	*Helianthus ciliaris* DC.	Heliantheae	+	0	+	0
Costunolide	*Ambrosia chamissonis* (Gray) Payne	Heliantheae	+	0	+	0
	Liriodendron tulipera L.	Family Magnoliaceae			0	0
Dehydroartemorin (Anhydro verlotorin)	*Artemisia verlotorum* Lamotte	Anthemideae	+	0	+	0
Dentatin B	*A. tridentata* Nutt. ssp. *tridentata*	Anthemideae	+	0	+	0
Eriofertin	*Eriophyllum confertiflorum* (DC) Gray	Heliantheae	+	0	+	0
Eupacunin	*Eupatorium cuneifolium* (Tourn.) L.	Eupatorieae	+	0	+	0
Jurineolide	*Jurinea albicaulis* Bge. var. Kilae	Cynareae	+	0	0	0
Laserolide	*Laserpitium siler* L.	Family Umbelliferae	—	0	0	0
Laurenobiolide	*Laurus nobilis* L.	Family Lauraceae			0	0
	The de-acetyl compound, chamisellin, is present in *Ambrosia chamissonis* q.v.	Heliantheae				
Novanin	*Artemisia nova* Nels.	Anthemideae	+	0	+	0
Orizabin	*Tithonia tubaeformis* Jacq. Cass.	Heliantheae	+	0	0	0
Tatridin-A, B and C	*Artemisia tridentata* Nutt. ssp. *tridentata*	Anthemideae	+	0	+	0
Tomentosin	*Montana tomentosa* Cerv.	Heliantheae	+	0	0	0
Tulipinolide	*Liriodendron tulipera* L.	Family Magnoliaceae	+	0	0	0
	The de-acetyl compound, chamissarin, is present in *Ambrosia chamissonis* q.v.	Heliantheae				
Zexbrevin	*Zexmenia brevifolia* Gray	Heliantheae	+	0		0
Zexbrevin B and C	*Zexmenia brevifolia* Gray.	Heliantheae	+	0	0	0
Class Guaianolide						
Acetyliso-montanolide	*Laserpitium siler* L.	Family Umbelliferae	—	0	0	0
Artefransin	*Artemisia franserioides* Greene	Anthemideae	+	0	+	0

TABLE I—(cont.)

			Immunological activity			
			Chemical compound			
Chemical compound	Botanical source	Tribe	Potential*	Reported	Genus	Species
Arteglasin, A and B	*A. douglasiana* Bess	Anthemideae	+	0	+	0
Canin	*A. cana* Pursh ssp. *cana*	Anthemideae	+	0	+	+
8-Deoxycumambrin	*A. nova* Nels; *A. tripartita* Rydb. ssp. *rupicola* Beetle	Anthemideae	+	0	+	0
Isomontanolide	*Laserpitium siler* L.	Family Umbelliferae	—	0	0	0
Montanolide	*Laserpitium siler* L.	Family Umbelliferae	—	0	0	0
Class Elemanolide						
Confertiphyllide	*Eriophyllum confertiflorum* (DC) Gray	Heliantheae	+	0	+	0
Class Pseudoguaianolides						
Bipinnatin	*Parthenium bipinnatifidum* (Ort.) Rollins	Heliantheae	+	0	+	0
Chiapin A and B	*Parthenium fructicosum* Less.	Heliantheae	+	0	+	0
Incanin	*Parthenium tomentosum* DC.	Heliantheae	+	0	+	0
Ivoxanthin	*Cyclachaena xanthifolium* Nutt. ($\cong$ Iva)	Heliantheae	+	0	+	0
Neoambrosin	*Parthenium bipinnatifidum*	Heliantheae	+	0	+	0
	Hymenoclea salsola T. and G.	Heliantheae	+	0	0	0
Class Xanthanolide						
Acetylparthemollin	*Iva ambrosiaefolia* Gray	Anthemideae	+	0	+	0
Apachin	*Iva ambrosiaefolia*	Anthemideae	+	0	+	0
Ivambrin	*Iva ambrosiaefolia*	Anthemideae	+	0	+	0

+ Potential or reported allergenicity.
— Reported absence of allergenicity.
0 No report found.
* Presence of α-methylene group exocyclic to the γ-lactone.

Some authorities place *Ambrosia* in a tribe Ambrosiae and separate the tribe Helenieae from the Heliantheae. Here, and previously (Mitchell and Dupuis, 1971), we have included *Ambrosia* in the tribe Heliantheae and Helenieae in the Heliantheae, according to the classification of Willis (1966).[2]

The role of " secondary products " of plant metabolism is poorly understood; such products are not known to be necessary to the metabolism of the plants which produce them. Recently it has been reported that such lactones inhibit the sprouting of seeds of some other plants and are antibiotic for fungi, possibly therefore giving the " weeds " among the Compositae a preferential ecological position over man's economically more desirable plants (Dalvi *et al.*, 1971).

Up to the present time, we have observed 35 forest-workers with allergic contact dermatitis, caused by *Frullania nisquallensis* (a liverwort), masquerading as so-called " cedar-poisoning " in British Columbia. These patients also exhibit sensitivity to numerous sesquiterpene lactones, derived from Compositae species, such as alantolactone (Fig. 1, IV). Those of our 35 patients who have been so tested show negative patch test reactions to other compounds possessing an unsaturated methylene group, viz., l-protolichesterinic acid (Shibata, 1958), ilicic acid and α-methylene-γ-butyrolactone (Fig. 1). Evidently a lactone ring and a sesquiterpene attached to the lactone are immunochemical requisites in addition to a $=CH_2$ group for these patients.

Two patients with allergic contact dermatitis from *Frullania* have recently been observed in Portland, Oregon (Storrs, 1972). Bancons (1966, 1967) previously reported 75 such cases from France and there have been a few other case reports from Europe, none from the United Kingdom or North America.

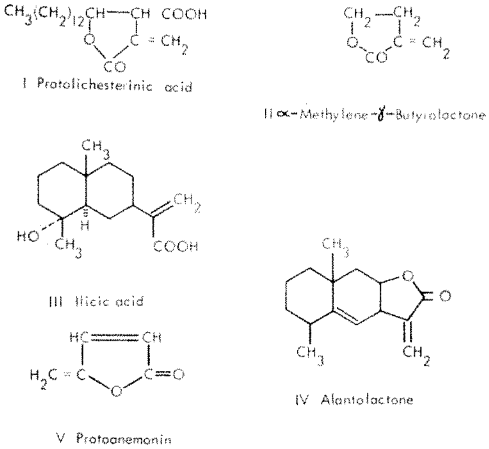

FIG. 1.—Chemical formulae of: I. Protolichesterinic acid (aliphatic lactonic acid) derived from *Cetraria* and *Nephromopsis* spp. (lichens); II. α-methylene-γ-butyrolactone (derived from *Tulipa*, family Liliaceae); III. Ilicic acid (sesquiterpenoid derived from *Ambrosia*, family Compositae); IV. Alantolactone (a representative allergenic sesquiterpene lactone derived from *Inula*, family Compositae, possessing a methylene group attached to the lactone); V. Protoanemonin (derived from buttercup, *Ranunculus* spp., family Ranunculaceae).

Of our 35 patients, 11 are also allergic to d-usnic acid derived from lichens (Mitchell and Shibata, 1969), probably by multiple specific sensitivity. We have not seen a patient who was allergic to chemical compounds derived from lichens who was not also allergic to *Frullania*.

Another unsaturated lactone derived from plants (*Actaea*, *Anemone*, *Caltha*, *Clematis* and *Ranunculus* spp., family Ranunculaceae) is protoanemonin (Fig. 1, V), which has strong primary irritant properties, producing sub-epidermal bullae in the skin (Burbach, 1963). Protanemonin is derived from ranunculin, which is present in the leaves of members of the buttercup family. There are many papers in the dermatological literature relating the irritant effect of such plants (the literature will not be reviewed here), and we have not tested protoanemonin for allergenic activity.

A sample of l-protolichesterinic acid was donated by Professor S. Shibata, Faculty of Pharmaceutical Sciences, University of Tokyo, Japan, and a sample of tulipalin (α-methylene-γ-butyrolactone was donated by Dr J. C. M. Beijersbergan, Laboratium voor Bloembollenonderzock, Lisse, Netherlands.

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SEBORRHOEIC DERMATITIS OF THE BEARD

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Summary.—Seborrhoeic dermatitis of the face is common in those males who grow moustaches or beards. Although the pathophysiology of the process remains hypothetical, the prevention and therapy are clear cut. They involve the use of shampoos containing tar, salicylic acid and sulphur and, in extensive cases, the use of topical corticosteroids. There is no indication for radical surgical procedures such as shaving.

TODAY many men have again chosen to display a uniquely masculine symbol—the beard. However, after enduring the awkward "growing out" stage, the self-conscious identity crisis, and the tireless comments of their colleagues, many then face another problem—a pruritic, erythematous scaling eruption of the beard area. The purpose of this communication is to point out that this very common eruption is seborrhoeic dermatitis and that its therapy does not necessitate shaving.

Seborrhoeic dermatitis of the moustache and beard areas arising concomitantly with facial hair growth was frequently mentioned in dermatology textbooks written during times of popularity of the beard (Buckley and Duncan, 1898; Ormsby, 1915; Whitfield, 1921). Duhring (1886) commented that early changes of "seborrhoea faciei" included sebaceous material protruding from hair follicles contaminated by collections of extraneous matter. A year later Unna discussed a scaly form of seborrhoeic dermatitis which appeared "when a beard is worn" (Unna, 1887).

Seborrhoeic dermatitis is a descriptive term for an erythematous, scaling eruption which occurs primarily in "seborrhoeic" areas—that is, those with a high number of active sebaceous glands such as eyebrows, nasolabial folds, scalp and presternal areas (Montagna, 1963; Calman *et al.*, 1970). The disease occurs in neonates and at the time following puberty when sebaceous glands are functioning most actively. However, a consistent, direct relationship between the amount or composition of sebum and the presence of dermatitis has not been documented. Moreover, Pochi (1972, personal communication) has observed that the administration of oestrogen to patients with seborrhoeic dermatitis in doses which decrease sebaceous gland secretion results in no significant improvement in the dermatitis.

The specific cause of seborrhoeic dermatitis is unknown. Reports which attribute aetiological importance to increased sugar consumption (Bett *et al.*, 1967), vitamin B malutilization (Casper and Manfredi, 1968), auto-immune phenomena (Hashimoto, 1966), or which attempt to correlate the disease with major blood groups (MacSween and Syme, 1965), have not been convincing. A constitutional predisposition exists, but its exact role is not defined. Emotional and physical stress may be important; seborrhoeic dermatitis has been noted to occur

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after myocardial ischaemia (Tager *et al.*, 1964). The dermatitis, as well as increased sebum output (Grasset and Brun, 1959), are common in Parkinsonism and other neurological diseases (Pochi *et al.*, 1962). Early reports that levodopa produced a fall in sebum excretion rate in Parkinsonian patients (Burton and Shuster, 1970; Appenzeller and Harville, 1970) were not confirmed in a study of 7 such patients by Cotterill *et al.* (1971), but treatment of Parkinson's disease with L-dopa has been noted to improve the coexisting seborrhoeic dermatitis (Parish, 1970). Even more intriguing are the reports of unilateral seborrhoeic dermatitis in syringo-myelia (Tronnier, 1964) and dermatomal seborrhoeic dermatitis associated with a lesion of the trigeminal nerve (Bettley and Marten, 1956).

The most frequent site of seborrhoeic dermatitis is the scalp—its mild form being simple dandruff. The eyebrow is another area often affected in both sexes. Erythema and scaling are accompanied by pruritus, which may be marked. The scale may be thick, semiconfluent, yellow to brown and greasy, or it may remain unimpressive, fine, white and dry. Although folliculitis and pyoderma are often present as secondary complications of seborrhoeic dermatitis (Beare *et al.*, 1968; Spoor, 1968), no specific bacterium, fungus or parasite has been shown to be of aetiological significance. The histological reaction pattern is not specific and has features of both psoriasis and chronic dermatitis. In psoriasiform tissue reactions, such as seborrhoeic dermatitis, engorged capillary tufts in the dermal papillae rhythmically discharge leukocytes and serum through temporary breaks in the basement membrane into the epidermis (Pinkus and Mehregan, 1966). The resulting inflammation is seen as intercellular oedema and parakeratotic scaling.

Males with no previous history of seborrhoeic dermatitis may begin to note some diffuse erythema and pruritus of the face within 2 to 3 days of uninterrupted hair growth. Both authors of this communication noted slightly pruritic erythematous greasy papules of the beard area by the third day of beard growth. Over the next several days this progressed to a pruritic, semiconfluent, raised, erythematous, dull yellow-to-red, dry scaling eruption of the area recently covered by hair. Involvement was most impressive in the hollows of the cheeks, where the rash extended slightly beyond the beard toward the zygomatic arch. One of us regrew his beard several times and noted the same sequence of events each time. However, if one side of the face was lathered daily, rinsed and doused with alcohol-containing aftershave, no dermatitis developed on that side, even although no razor was used. If the dermatitis was allowed to develop fully, it could be easily terminated by the application of topical corticosteroids.

We found that the entire process could be prevented by the twice-weekly use of an " antiseborrhoeic shampoo " and subsequently have been able to grow our own beards for over 2 years without symptoms or signs of seborrhoeic dermatitis. During that time we have seen many newly bearded men with a similar dermatitis which in some cases was complicated by folliculitis, pyoderma or impetiginization, despite good hygiene. All have responded to treatment for seborrhoeic dermatitis.

It is not clear why the beard shares the liability to seborrhoeic dermatitis with the scalp and presternal area. Why should the presence of hair make the face more susceptible to dermatitis? Most of the areas in which seborrhoeic dermatitis develops in the adult are hairy areas. That portion of the hair shaft beneath the surface of the skin may act as a wick emptying sebum and keratinous debris from hair follicles; the portion of shaft which protrudes above the razor line could function as a nidus for the collection of these products as well as surface debris and sweat. Decrease in mechanical surface cleansing on the bearded face, associated with the prolonged retention of sebum on the skin surface, may in fact initiate the inflammatory reaction. The sebum may act as a primary irritant or may alter epidermal function following percutaneous re-entry. Pooling of sebum and its reabsorption would be maximal in areas of apposition of skin folds. This may account for the frequent presence of dermatitis in the nasolabial, perioral, and postauricular creases on the face and in the intertriginous areas on the body. On the bearded face an additional factor is lack of ultra-violet light reaching the skin.

Prevention and treatment of seborrhoeic dermatitis of the beard area is relatively simple. The lather, surface scraping and rinsing which accompany daily shaving are usually followed by the application of an " after-shave " lotion containing alcohol. This combination of mechanical debridement with a mild lipid solvent inhibits the onset of dermatitis. When a beard is growing, good hygiene, including frequent shampooing, serves the same purpose. Once seborrhoeic dermatitis occurs in the beard, the milder or chronic forms can be controlled by shampoos containing tars, salicylic acid, sulphur and other proven anti-seborrhoeic topical agents. The pruritus, scaling and erythema associated with more extensive cases respond within days to the use of topical corticosteroids.

Duhring pointed out in 1886 that, in the treatment of seborrhoeic dermatitis, " concerning the cutting of the hair to facilitate the treatment, I would remark that the process is never necessary " (Duhring, 1886). In our experience this is certainly true of the beard. Since dermatological therapy is so successful, the bearded male should beware of those who would cure this itch with a radical procedure, for he may find himself, like Samson: " If I be shaven . . . I shall be like any other man " (Judges, 16 : 17)!

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DERMATITIS IN MAN AND IN A DOG CAUSED BY THE MITE *CHEYLETIELLA YASGURI* SMILEY

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Summary.—The authors have observed an itching dermatitis of 9 months duration in the 3 members of a family in the Netherlands. The source of the itch appeared to be a mangy dog that lived in contact with the family and was heavily infested by the mite *Cheyletiella yasguri* Smiley. All the symptoms disappeared quickly in both the family and the dog after treatment with an acaricide.

THE diagnosis of animal scabies in man is rarely made. However, infestations with animal ectoparasites seem to be of greater importance than previously was believed (Fain, 1968, 1969; Hewitt *et al.*, 1971).

The morphological diagnosis is less easy than in human scabies, in which there is such a typical clinical picture, for animal scabies may evoke a wide diversity of cutaneous responses in man.

When several members of a family complain of an itching eruption and there is also a scratching pet animal, examination of this animal will probably provide the key to the solution of the problem.

CASE REPORT

A family consisting of father aged 53, mother aged 31, and daughter aged 8, living in Rotterdam, came to the out-patients' department of Dermatology, all complaining of severe itching on the trunk for 9 months.

The daughter was the first to complain, but soon both father and mother also developed severely pruritic eruptions, which began at approximately the same time. The family owned a dog (a boxer) which had suffered from " dandruff " for 5 months and had been scratching for 9 months. The patients were treated by the family doctor with creams and a diet. A dermatologist who was consulted prescribed ointments and tablets, but the symptoms persisted. During a holiday (without the dog) in the Canary Islands they were totally free of symptoms; on their return home, the itching and skin lesions re-appeared. The dermatologist discovered parts of mites in material from a lesion and treated the family with an acaricide, but after 3 or 4 weeks new lesions appeared. On examination at our clinic the findings were:

Mother: anterior region of the trunk, papules surrounded by slight erythema; buttocks, papules, excoriations and hyperpigmented macules.

Daughter: abdomen, some excoriated papules; buttocks, papules and excoriations; dorsum of the left hand, 2 papules.

Father: anterior region of the trunk, and buttocks, papules and excoriations.

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Burrows, as in human scabies, were not found. We could not demonstrate mites in scrapings from the lesions. Skin scrapings from the dog consisted of crusts, hair and many living mites (just visible with the naked eye). Under the dissecting-microscope we saw mites (Fig. 1), different from *Sarcoptes scabiei* (Fig. 1).

The dog was treated by a veterinary surgeon with gamma benzene hexachloride lotion and, after 4 days, was free of symptoms. The family was treated with benzyl benzoate emulsion and, after 1 week, they were free of itching and skin lesions. The interior of the house was sprayed with an insecticide.

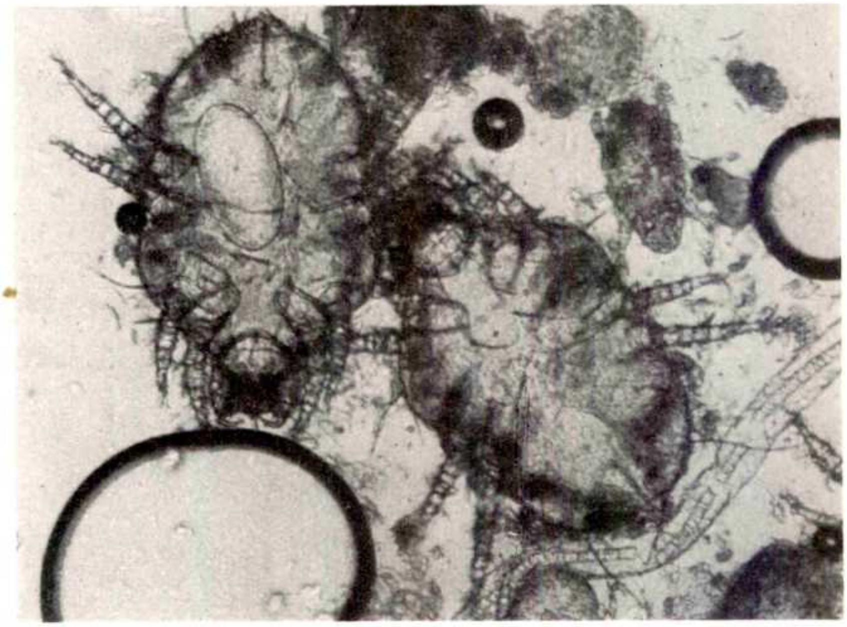

FIG. 1.—*Cheyletiella yasguri* Smiley. Females (one gravid) in ventral view.

We observed that these mites could survive for almost 2 days away from the host; the last living mite was seen after 42 hours. The mites found on the dog were identified by one of us (A.F.) as *Cheyletiella yasguri* Smiley.

DISCUSSION

The mite *Cheyletiella yasguri* (Smiley, 1965) was described in the U.S.A. The type specimens were collected from Schnautzer pups in Mamaroneck, New York, in 1963. The mites had produced a superficial scurf at the base of the tail. Other specimens were also found on an adult mangy dog at Cornell University, Ithaca, New York, in 1962. The species has been named after Dr I. Yasgur, who found the mites.

Kuscher (1940) and Kutzer (1963) have recorded mange in dogs produced by a related species, *Cheyletiella parasitivorax* (Megnin, 1878).

Cheyletiella parasitivorax is a common and cosmopolitan parasite of the fur of rabbits. It has been found occasionally on cats, in which animal it may be associated with mange. Several cases of human itching dermatitis have been recorded in persons in contact with such infested cats. In all these cases the itching disappeared when the animals had been treated with acaricides (Baker *et al.*, 1956).

Recently Rack (1971) has recovered *Cheyletiella yasguri* from 2 mangy dogs in Germany. The owners of these dogs showed an itching dermatitis which was attributed to contact with the parasitized animals. Rack surmised that all the cases of mange in cats, dogs and men recorded so far in the literature and attributed to *Ch. parasitivorax*, were in fact caused by *Ch. yasguri*.

Our observation in the Netherlands provides a further example of the pathogenicity of *Ch. yasguri* for dogs and men.

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DEFICIENT FOLATE ACTIVITY DURING TREATMENT OF PSORIASIS WITH METHOTREXATE DIAGNOSED BY DETERMINATION OF SERINE SYNTHESIS IN LYMPHOCYTES

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Summary.—Deficient folate activity was diagnosed in all of 7 patients treated for psoriasis with methotrexate (Mtx) by applying a new method (Ellegaard and Esmann, 1972a) of assaying folate activity which utilizes the folate-dependent incorporation of ^{14}C-formate into serine of lymphocytes during incubation under standardized conditions. The arithmetical mean of this folate activity was 4·8% ± 0·6 (SEM) of added ^{14}C-activity per 10^9 lymphocytes per 4 hours, as compared to 9·2% ± 0·4 in 24 normals. The ^{14}C-formate incorporation into RNA was severely depressed in each case. Mtx added to lymphocytes *in vitro* had no effect on the folate activity.

In addition, the new observation was made that patients under treatment with Mtx develop decreasing serum concentrations of B_{12}, possibly due to an impaired B_{12}-absorption.

TREATMENT of psoriasis with folate antagonists (Gubner *et al.*, 1951), mainly methotrexate (Mtx), is accompanied by a series of side-effects, which may be attributed to folate deficiency, notably megaloblastic anaemia, diarrhoea and liver cirrhosis (Berge *et al.*, 1970). In addition, extensive loss of folate may occur in exfoliative skin diseases (Hild, 1969). Mtx (4-amino-N^{10}-methyl-4-deoxyfolic acid) exerts its influence by an inhibition of folate reductase (Osborn *et al.*, 1958), preventing the normal reduction of dihydrofolic acid to the metabolically active tetrahydro-form.

A measurement of the concentration of folate in serum and whole blood by the *L. casei* method usually employed is not possible under Mtx treatment because the growth of the test micro-organism is completely inhibited by the drug (Klipstein, 1964; Waters and Mollin, 1961). A new method developed by us (Ellegaard and Esmann, 1972a), however, circumvents this problem by measuring folate activity as the rate of ^{14}C-formate incorporated into serine by the patient's own lymphocytes. The method has been applied with success to patients with folate deficiency due to malnutrition and malabsorption (Ellegaard and Esmann, 1972b), to disclose folate deficiency in pernicious anaemia (Ellegaard and Esmann, 1972c), and to investigate the action of pharmacologically active drugs on the folic acid metabolism of lymphocytes (Ellegaard and Esmann, 1972d).

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TABLE 1.—*Haematological Data and Lymphocyte Folate Activity of 7 Patients treated with Mtx. For Normals. The Mean ± SEM is Given Together with the Range of Observed Values*

	Patients							Normal values $n = 24$	
	M.J.	O.L.	J.K.	A.L.	A.B.O.	M.C.	J.S.J.	Mean	Range
Duration of treatment, weeks	56	21	60	43	10	10	23		
Total dose of Mtx, mg	650	285	900	450	140	100	235		
Hb, g/100 ml	10·5	14·2	13·5	12·7	13·4	15·4	12·9	♂15·4±0·3 ♀13·6±0·2	
Serum-iron, μg/100 ml	123	179	49	124	65	154	67	>80	
Serum-B_{12}, pg/100 ml	150	260	180	200	510	510	440	>200	
Schilling test, %	3	8	11	21	23	15	28	>9	
Hypersegmented PMN, %	34	22	2	8	6	1	5	0·7±0·2	0–3·0
Lobe average	4·5	4·4	3·2	3·9	3·9	2·8	3·4	3·1±0·07	2·4–3·8
Bone Marrow	nb(a)	nb	nb	nb	slightly mb(b)	nb	—		
Serum-folate, ng/ml	t.i.(c)	t.i.	0·8	t.i.	t.i.	t.i.	t.i.	5·0±0·6	2·3–14·4
Whole blood folate, ng/ml	t.i.	t.i.	20·5	t.i.	t.i.	t.i.	t.i.	78·4±5·8	
FIGLU test, μmol/hour	235	16	37	52	58	4200(d)	122	0	
Incorporation/10^9 lymph./4 hours (% of added ^{14}C-activity)									
Serine(e)	5·4	5·1	5·7	4·9	4·5	4·1	4·1	9·2±0·4	6·3–14·3
Methionine	1·4	1·0	1·4	2·1	1·2	1·7	0·7	2·1±0·2	1·0–4·6
RNA	0·2	0·6	1·1	0·6	0·2	0·3	1·0	2·7±0·2	1·1–4·7
Protein	0·4	0·3	0·9	0·2	0·7	0·9	0·5	0·7±0·07	0·3–1·1

(a) Normoblastic = nb; (b) megaloblastic = mb; (c) total inhibition = t.i.; (d) μmol/l, urine volume unknown, (e) ^{14}C-formate incorporation in serine = " folate activity ".

MATERIAL AND METHODS

Material

The material consisted of 7 patients with psoriasis treated with Mtx for varying periods of time and a group of 24 healthy control subjects, 12 men and 12 women, aged 20–85 years. Haematological data of the 7 patients, obtained by routine laboratory methods, are apparent from Table I. No variation attributable to sex or age, except for haemoglobin (Hb) and haematocrit, was observed in the data of the control group.

Methods

Lymphocytes were separated from whole blood of fasted donors, as previously described (Hedeskov and Esmann, 1966), and 30×10^6 lymphocytes were suspended in 5 ml Krebs-Ringer-bicarbonate buffer, pH 7·46, supplemented with 6·7 mmol glucose, 20 mmol glycine, 1% gelatine and 10 μCi ^{14}C-formate (59 mCi/mmol). The lymphocyte suspensions were contaminated by 0·6% ± 0·06 of polymorphonuclear leukocytes and by 4·0 ± 0·1 erythrocytes per lymphocyte, an admixture which was found negligible. Duplicate incubations of the lymphocytes were carried out at 37°C for 4 hours in flasks closed with rubber caps and under an atmosphere of 95% O_2 and 5% CO_2. The results of an examination of the ability of lymphocytes to survive under these conditions has previously been published (Hedeskov and Esmann, 1966). The incubation was terminated by injecting 0·5 ml 33% perchloric acid through the rubber cap. The flasks were then placed in an ice bath for 1 hour and the acid insoluble material (protein, lipids, RNA and DNA) separated by centrifuging at 1800 *g* for 10 min.

The amino-acids in the supernatant were separated on Dowex 50 H^+ and eluted by 1·5 N NH_4OH, which was evaporated. The ^{14}C-incorporation into serine was determined by periodate degradation (Sakami, 1955) and 98% of the activity was found in the C_3-position. Recovery of this carbon of serine was 96·8% ± 1·5 in 50 degradations. In preceding experiments the optimum conditions for the assay were established (Ellegaard and Esmann, 1972a). It was found that serine synthesis of the lymphocytes was proportional to the incubation time within 4 hours, and to the number of lymphocytes incubated, for the range employed. Furthermore, it was found that incorporated ^{14}C-formate in serine was proportional to the added amount of labelled formate and the specific activity thereof. Maximal serine synthesis by the lymphocytes was found with 6·7 mmol glucose and 20 mmol glycine in the buffer.

The ^{14}C-activity incorporated is expressed as the percentage of added ^{14}C-formate per 10^9 lymphocytes per 4 hours (" folate activity ").

Under the same experimental conditions the ^{14}C-incorporation in methionine, RNA and protein was also followed and is expressed in a similar way, but the ^{14}C-formate incorporation into these products does not proceed under optimal conditions. The incorporation of ^{14}C into methionine was routinely determined after isolating methionine by paper chromatography in phenol/water.

Protein and RNA were separated from the perchloric acid insoluble material by a modification of the method of Schmidt and Thannhauser (1945). Residual ^{14}C-formate was removed from the precipitate by washing with 3×1 ml ice-cold 3% perchloric acid containing 0·2% sodium formate. Lipids were extracted by subsequent treatment of the precipitate with ethanol/ether (3 : 1) and ether. The dried residue was dissolved and RNA hydrolysed in 1 ml potassium hydroxide at 37°C for 12 hours. The solution was neutralized with 3% perchloric acid, placed in an ice bath for 30 min and the precipitated potassium perchlorate removed by centrifugation. Protein and DNA were then precipitated from the supernatant by adding perchloric acid to a final concentration of 3% and separated by centrifugation. The residue was dissolved in 1 ml Hyamin 10-X at 37°C and the ^{14}C-activity of 50–100 μl samples counted in a scintillation counter. As no DNA is synthesized by the lymphocytes during 4 hours incubation, this radioactivity is due to ^{14}C-activity incorporated in the protein. The RNA-mononucleotides were obtained from the supernatant of the last precipitation. Perchloric acid was removed as the potassium salt and, after evaporating the RNA solution in a vacuum extractor at 40°C, the RNA-mononucleotides were dissolved in 100 μl distilled water and the ^{14}C-activity in samples of 25 μl counted by liquid scintillation.

The folate concentration of serum or whole blood was determined by a microbiological assay with *L. casei* after extraction under protection of ascorbic acid. Erythrocyte folate was calculated according to Cooper and Lowenstein (1964). The B$_{12}$-concentration of serum samples was determined by a *L. leichmannii* assay.

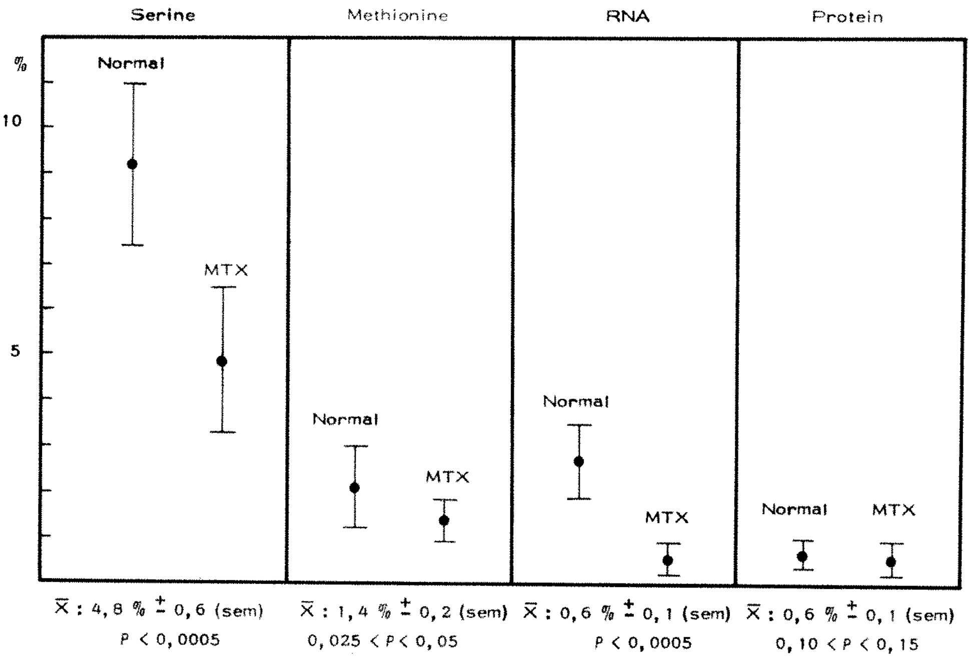

FIG. 1.—Incorporation of ^{14}C-formate in % of added activity in 24 normals and 7 patients with psoriasis treated with methotrexate (Mtx). The arithmetical mean ± 1 × SD is given. Below each column the mean ± SEM for the values obtained on the patients is given, together with the probability of identity to the group of normals (Student's *t*-test).

Counting Procedures and Chemicals

Liquid scintillation counting was performed in the solvent system given by Rapkin (1963) with a Packard Liquid Scintillation Counter (Tri-carb 2002/2009), efficiency for ^{14}C-counting 79·8%, to an accuracy of 1%. Quench corrections were done by the Channels ratio method (Herberg, 1965).

All chemicals were of analytical grade. Dextran-150 was from Pharmacia, Sweden, and Dowex-50, 8 × mesh 100–200, from Sigma, U.S.A. Methotrexate was from Lederle, U.S.A., and ^{14}C-formate from the Radiochemical Centre, England.

RESULTS

The weekly dose of Mtx varied little from patient to patient, and there is therefore a close correlation between the duration of treatment and total dose of Mtx (Table I). The haematological data show that one patient (M.J.) was anaemic, 3 (J.K., A.B.O., J.S.J.) had a low concentration of serum-iron, and one patient (M.J.) had a borderline value of the serum-B_{12}-absorption. The polymorphonuclear (PMN) leukocytes were hypersegmented, with increased lobe average in 4 cases, and all patients had increased urinary excretion of formiminoglutamic acid after oral administration of l-histidine (FIGLU test). The concentration of folate in serum and whole blood could not be determined in 6 of the 7 cases because of complete inhibition of the growth of the test microorganism (*L. casei*).

The determination of folate activity in lymphocytes by measuring the incorporation of ^{14}C-formate into serine showed this to be far below the normal range in each case. The simultaneously measured incorporation of ^{14}C-formate into other products showed for RNA also in each case a pronounced decrease, whereas the incorporation into protein and methionine was normal or only slightly decreased respectively (Fig. 1).

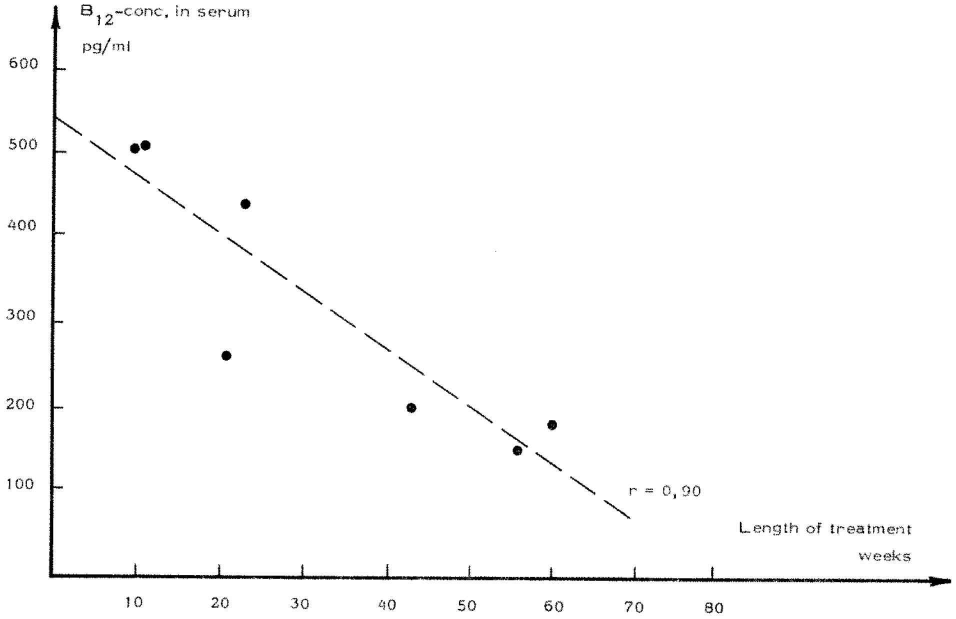

FIG. 2.—The level of the B_{12}-concentration in serum from 7 patients with psoriasis treated with methotrexate as a function of the length of this treatment.

Besides the demonstration of a deficient folate function, an analysis of the results suggests a correlation between the duration of treatment and the serum concentration of vitamin B_{12} (Fig. 2).

In connection with the described effect of Mtx *in vivo*, it was also found of interest to study its action *in vitro*. It has previously been found (Kessel *et al.*, 1968) that lymphocytes accumulate insignificant amounts of activity from tritiated ametopterine, whereas PMN leukocytes take up labelled amethopterine to a much higher degree. For this reason PMN leukocytes instead of lymphocytes were applied to the study detailed in Table II, which shows that Mtx *in vitro* has no

TABLE II

		^{14}C-formate incorporation (% of control)		
Exp.	Mtx (μmol)	Serine	RNA	Protein
3	1·0	91	84	84
2	2·5	86	—	—
1	5·0	93	—	—
3	10·0	93	85	(50)
2	10·0	92	—	—
2	100·0	96	105	111
3	100·0	104	—	—
4	100·0	100	118	100
4	1000·0	102	86	100

Ten to 15 × 10^6 PMN leukocytes/ml were incubated for 4 hours in 10 ml Krebs-Ringer bicarbonate buffer with 1% gelatine, 6·7 mmol glucose, 10 μCi ^{14}C-formate, and with or without methotrexate (Mtx) in the stated concentrations. Mtx was dissolved in 50–100 μl 2·6 mmol NaOH and added to the cells for a 20 min preincubation before addition of ^{14}C-formate. Experiments with the same number were performed with the same batch of cells and the same control incubation. In exp. 1 and 2 serine was isolated by paper chromatography (Ellegaard and Esmann, 1972a) and ^{14}C-activity registered by liquid scintillation counting after elution of the serine. In exp. 3 and 4 serine was determined by degradation; cf. Methods.

effect on the folate activity of the cells as expressed by the rate of the folate-dependent incorporation of formate into serine. The ^{14}C-labelling of RNA and protein was also unaffected by Mtx for a wide concentration range of this drug in the incubation buffer.

DISCUSSION

The present investigation has shown that a folate-dependent activity of lymphocytes from patients treated with Mtx can be measured and that this activity was reduced to half the arithmetical mean after Mtx treatment. The suppression of folate activity was also evident from the strongly inhibited incorporation of ^{14}C-formate into RNA, whereas the incorporation of ^{14}C-formate into methionine was not as severely depressed. The reason for this is not quite evident. First of all, the experimental conditions are not such as to maximize methionine synthesis, and a greater variability of the results is to be expected, but it is also possible that a contributory factor could be the need of the cells to regenerate tetrahydrofolate from the N^5-methyl-derivative by methionine synthesis. It has been found (Waxman *et al.*, 1969) that the inhibitory effect of Mtx on folate-dependent reactions is partly reversed by homocystein, the substrate for methionine synthesis. The inhibition of RNA synthesis might explain the general inhibition of enzymes of carbohydrate metabolism seen in the epidermis of patients with psoriasis treated with Mtx (Halprin and Ohkawara, 1967).

One of the advantages of the present method for the determination of folate activity is that it can be applied to patients treated with Mtx. The growth of the test microorganism (*L. casei*) usually employed to estimate the folate concentration in serum or whole blood samples is in most cases totally inhibited by Mtx. In addition, this inhibition is present until 7 weeks after discontinuation of the treatment because of a firm binding of Mtx to dihydrofolate-reductase, the enzyme responsible for the conversion of dihydrofolate to the active tetrahydrofolate form (Cooper and Lowenstein, 1964). Secondly, lymphocyte folate activity has been shown to react to folate deprivation within 4 weeks (Ellegaard and

Esmann, 1972b), whereas the FIGLU test requires 2–3 months to become abnormal (Herbert, 1962).

A hitherto unpublished effect of Mtx is to lower the serum concentration of vitamin B_{12}, perhaps due to impaired absorption of vitamin B_{12}. This effect might be related either to a topical effect of Mtx, resulting in jejunal mucosal atrophy (Sherlock *et al.*, 1962; Trier, 1962), or to the Mtx-induced impairment of the folate function itself, which leads to atrophic changes in the intestinal epithelium. The latter mechanism would then be a parallel to the impaired B_{12}-absorption or the decreased serum- and erythrocyte B_{12}-levels observed in various other cases of folate deficiency (Harrison, 1971; Omer *et al.*, 1970; Scott *et al.*, 1968).

Mtx added to PMN leukocytes had no effect on the folate activity of these cells, which is consistent with the observations of Winzler (Winzler *et al.*, 1957, 1959), who found that Mtx uptake took place only in immature cells.

The measurement of folate activity in lymphocytes might be of value in the assessment of patients developing resistance to folate antagonists. A simplified version of the method for measuring folate activity is under preparation (Sølling *et al.*, 1972).

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EFFECT OF RETINOIC ACID IN PSORIASIS, II

LONG TERM STUDY

ANGUS MACDONALD, R. M. H. McMINN AND LIONEL FRY

Summary.—Clinical and histological features and mitotic counts have been studied in the lesions of 11 patients with psoriasis who were treated with topically applied retinoic acid ointment for a period of 3 months.

Serial biopsies were taken at monthly intervals from treated lesions in all 11 patients and from lesions treated with the unmedicated ointment base in 9.

There was no significant difference between treated and placebo groups regarding any of the parameters studied. Irritant effects of the retinoic acid were prominent in 7 patients.

The significance of these results is discussed in the light of the known mechanism of action of retinoic acid.

AN increasing number of reports have been appearing concerning the use of retinoic acid in the treatment of various skin diseases, including psoriasis (Frost and Weinstein, 1969).

We ourselves have recently reported the details of our findings concerning the response of psoriatic epidermis to retinoic acid applied topically over a period of 2 weeks (Fry *et al.*, 1970). This study demonstrated that the predominant effect of retinoic acid on psoriatic epidermis after 2 weeks was to be found in the granular layer, which is either reformed or becomes noticeably more prominent. Simultaneously there was a small but insignificant fall in the mitotic count and slight clinical improvement.

These findings, particularly the granular layer reformation, which have been observed by other workers (Frost and Weinstein, 1969) were interpreted by us as suggesting that retinoic acid exerted a " normalizing " effect on epidermal keratinization in psoriasis such that the parakeratosis so characteristic of the disease was reduced.

The changes found in the short-term study were sufficiently interesting to stimulate us to conduct a second study along similar lines, but over a longer period (3 months), to determine whether the clinical and histological effects of the drug would be maintained or bettered and thus to establish the therapeutic role of retinoic acid by itself in psoriasis.

PATIENTS AND METHODS

Patient Studies

Eleven patients with psoriasis were studied. No therapeutic agents were applied to their lesions for at least 2 weeks prior to the study, other than soft paraffin ointment, which was used if the lesions became fissured or painful.

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Retinoic acid ointment was applied twice daily to a number of lesions and the ointment base (of identical colour) was applied to other " control " lesions (which, as far as possible, were symmetrical and opposite).

The initial concentration of the ointment (which was stable under storage conditions and at 37°C) was 0·1% in all cases, but the irritant effect of the ointment necessitated weakening the concentration to 0·05% or 0·025% in many patients. The change to the weaker strength was made according to the individual patient's tolerance.

Clinical Results

The lesions were assessed clinically in terms of palpability, scaling and erythema; each of these features was given a score of 0, 1 or 2. (This method gives a typical untreated, thick, red, scaly plaque a score of 6, and normal-looking skin a score of 0).

Biopsies

Punch biopsies 3 mm in diameter were taken from the psoriatic lesions, using 2% lignocaine as local anaesthetic. Biopsies were taken from all the 11 patients whose lesions were treated with retinoic acid ointment, and from control lesions in 9 patients. The biopsies were taken before treatment and at 4, 8, 12 weeks and fixed in 10% formol-saline; paraffin sections were prepared in the usual manner and stained with H. and E. for histological studies.

Mitotic Counts

Mitosis in the epidermis was assessed by counting the number of mitotic figures in 100 unit lengths of the 3 basal layers of epidermal cells (unit length being arbitrarily taken as the diameter of one high power microscope field (× 320)). The counts refer to unit lengths of the layer immediately above the dermo-epidermal junction, not to unit lengths of epidermis.

Granular Index

The state of the granular layer was assessed by means of an arbitrarily defined " index " in which a score of 0 = absent granular layer; 1 = thin granular layer in small areas; 2 = granular layer in approximately half the specimen; 3 = complete granular layer.

RESULTS

Clinical

Although there appeared to be a greater improvement in the treated than the placebo lesions, this did not achieve statistical significance (Table I). Of interest,

TABLE I.—*Clinical Assessment*

Patient no.			1	2	3	4	5	6	7	8	9	10	11	Mean
Before		A	6	6	6	6	6	6	5	6	6	6	6	5·9
		P	6	6	6	6	6	6	5	6	6	6	6	5·9
Weeks of treatment	4	A	4	4	3*	4	4	4	2	4	4	2	2½	3·4
		P	6	4	5	6	6	6	4	6	4	2½	3	4·7
	8	A	3	3	3½	6*	2½†	4	0	1½	2	1½	1½	2·96
		P	6	1½	6	6	5	5	3	6	2	2½	3½	4·23
	12	A	6	—	3	6	4	4†	0*	1½	1	0	3	2·85
		P	6	—	6	6	5	6	0	3	1½	0	6	3·85

Concentration of retinoic acid ointment reduced to:

A = Lesions treated with active (retinoic acid) ointment.
P = Lesions treated with placebo ointment.
† = Concentration of retinoic acid ointment reduced to 0·05%.
* = Concentration of retinoic acid ointment reduced to 0·025%.

however, was the observation that, at the end of the experimental period, the mean difference between the treated and untreated groups, which was apparent initially, had almost disappeared. It also seemed that, when the concentration of the retinoic acid was reduced (in order to reduce its irritancy), the clinical effect was reduced to a similar or greater extent.

Histological Features

There was no obvious overall change in the morphology of the epidermis over the period of assessment. This finding was in distinct contrast to the previously reported changes found at 2 weeks (Fry *et al.*, 1970).

Mitotic counts.—There was a definite fall in the mean epidermal mitotic counts, but this applied equally to both treated (30·4 before, 21·2 after) and the placebo areas (32·0 before, 20·3 after) (Table II).

TABLE II.—*Granular " Index "*

			Weeks of treatment					
Patient	Before treatment		4		8		12	
no.	A	P	A	P	A	P	A	P
1	2	0	0	0	0	0	—	—
2	3	2	3	3	3	2	—	—
3	2	—	3*	—	3	—	2	—
4	0	—	—	—	0†	—	1	—
5	1	—	1	—	3*	—	3	—
6	0	0	2	1	2	2	1*	0
7	—	—	2	—	3	—	3†	—
8	—	—	2	2	3	3	3	3
9	1	1	1	2	2	2	3	3
10	0	0	3	3	3	3	3	3
11	1	1	3	2	3	1	1	0
Mean	1·1	0·7	2·0	1·9	2·3	1·9	2·2	2·0
S.D.	0·8	1·0	1·1	1·1	1·2	1·1	1·0	1·5
S.E.	0·3	0·4	0·4	0·3	0·4	0·4	0·3	0·6

Concentration of retinoic acid reduced to:
A = Lesions treated with active (retinoic acid) ointment.
P = Lesions treated with placebo ointment.
† = Concentration of retinoic acid ointment reduced to 0·05 %.
* = Concentration of retionic acid ointment reduced to 0·25 %.

Granular index.—The increase in the granular layer, as judged by the granular index, was similar in pattern to the mitotic counts (Table III). There was a definite increase in the granular index in both the treated (1·1 before, 2·2 after) and placebo areas (0·7 before, 2·0 after).

Side Effects

Only 4 of the 11 patients studied tolerated the full strength ointment for the duration of the study. In one, the study had to be prematurely ended; one patient defaulted; and in 5 the strength of the preparation had to be reduced (as shown in Tables I, II, III). The nature of the irritant reaction was of interest. In some patients it took the form of a diffuse erythema with moderate discomfort (producing a reaction clinically identical to that following vigorous application of dithranol).

TABLE III.—*Mitotic Counts*

Patient no.	Before treatment		Weeks of treatment					
			4		8		12	
	A	P	A	P	A	P	A	P
1	52	24	27	24	24	23	—	—
2	48	38	21	44	26	29	—	—
3	11	—	20*	—	15	—	36	—
4	6	—	—	—	29*	—	37	—
5	15	—	31	—	48†	—	14	—
6	47	25	23	28	25	39	18†	30
7	—	—	12	—	0	—	4*	—
8	—	—	54	27	16	16	9	18
9	31	32	24	7	34	34	10	2
10	30	37	4	20	5	5	6	2
11	34	36	21	28	40	35	57	56
Mean	30·4	32·0	23·7	25·4	23·8	25·9	21·2	20·3
S.D.	16·9	6·2	13·4	11·0	14·3	12·1	18·1	20·4
S.E.	5·6	2·5	4·1	4·2	4·3	4·6	6·0	8·3

Concentration of retinoic acid reduced to:

A = Lesions treated with active (retinoic acid) ointment.
P = Lesions treated with placebo ointment.
* = Concentration of retinoic acid ointment reduced to 0·025%.
† = Concentration of retinoic acid ointment reduced to 0·05%.

In others the reaction was noticeably peri-follicular, associated with intense local pruritus and occasionally with an apparent spread of the psoriatic process (particularly around the affected follicles) in a Koebner-like manner. This effect settled slowly when application was stopped.

DISCUSSION

The results obtained in this study show that, when retinoic acid is applied in the manner described, it is of little therapeutic value. There was an improvement in both clinical and histological features, but this applied equally to the placebo-treated lesions. This effect can have various explanations, such as a systemic effect, due to percutaneous absorption, or a " sympathetic " response in the control lesions. Perhaps the most likely explanation is the well known sensitivity of the lesions of psoriasis to the beneficial effects of placebo treatment with bland preparations. A significant fact to emerge from this study was that the improvement which occurs in the clinical and microscopic features over 2 weeks (Fry *et al.*, 1970) is not maintained when retinoic acid is applied for a longer period of time. This observation must surely be explained in terms of the known dual action of the vitamin. The effect on keratinization, whereby a granular layer is reformed, is likely to be beneficial in psoriasis and seems to be the dominant action when the treatment time is short. However, when the drug is applied for a longer period, the irritant effect seems to predominate.

The mode of action of retinoic acid is still speculative. However, it seems likely that the irritant effects, at least, are precipitated by the action of the substance on intracellular lysosomal membranes; these are " destabilized ", their contents, mainly enzymic, released, and an inflammatory process initiated (Reid and Jarret, 1967). It would seem logical to use retinoic acid in conjunction with an anti-inflammatory agent such as a topical corticosteroid in order to reduce its irritant effect without diminishing the beneficial effect on keratinization. A study along these lines (Macdonald and Fry, 1972) has been undertaken and confirms the possible value of such a combination.

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STUDIES ON LOW DOSE CHLOROQUINE THERAPY AND THE ACTION OF CHLOROQUINE IN SYMPTOMATIC PORPHYRIA

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Summary.—Eight patients with symptomatic porphyria (porphyria cutanea tarda symptomatica, PCT) were treated with small doses of chloroquin sulphate over periods ranging from 2 to 16 weeks. A good clinical and biochemical response was obtained in all but one patient, who relapsed after initial improvement. Manifestations of acute untoward reaction were seen in one patient only. It is concluded that low dose chloroquine therapy is both safe and effective in PCT.

Studies in porphyric rats indicate that the mechanism of action of chloroquine in PCT is probably not related, as has been suggested, to the formation of a chloroquine-porphyrin complex which damages liver cells and thereby leads to a reduction in hepatic porphyrin stores.

Chloroquine therapy led to increased urinary iron excretion and an increase in the desferrioxamine-chelatable iron in patients with PCT. It is suggested that the prolonged beneficial effects of chloroquine treatment may be related to reduction in a specific pool of liver iron.

It is well recognized that administration of the 4-aminoquinoline, chloroquine, to patients with symptomatic porphyria (porphyria cutanea tarda symptomatica, PCT) leads to a marked increase in urinary excretion of uroporphyrin (Sweeney *et al.*, 1962, 1965; Cripps and Curtis, 1962; Felsher and Redeker, 1966; Vogler *et al.*, 1970). The uroporphyrinuria is accompanied by an acute reaction lasting several days and characterized by fever, malaise, anorexia, nausea and moderate upper abdominal pain. Serum transaminase levels increase markedly (Sweeney *et al.*, 1962), and centrilobular liver cell necrosis has been observed at the time of maximum transaminase elevation (Felsher and Redeker, 1966).

The subsequent clinical and biochemical improvement noted in these patients prompted trials of chloroquine therapy in PCT (Sweeney *et al.*, 1965; Felsher and Redeker, 1966). The severity of the acute reaction in some patients on daily doses of 0·5 to 1·0 g was disturbing. Saltzer *et al.* (1968) investigated the possibility of employing a more protracted low dose regime. They reported beneficial effects without untoward reaction in a single patient given 0·5 g chloroquine twice weekly for 6 months. The present study describes our experience with 8 patients treated similarly and provides further information on the action of this drug in PCT.

MATERIALS AND METHODS

Patient Study

Seven adult African male patients admitted to King Edward VIII Hospital, Durban, and one white adult male patient (A.K.), who was referred by a private practitioner, volunteered for the study. Clinical features of PCT in these patients, together with

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relevant biochemical details, are summarized in Table I. All 8 patients admitted to long standing abuse of alcohol, and showed evidence of hyperpigmentation and scarring in areas exposed to sunlight. Hypertrichosis of the forehead was noted in 7 cases.

Fresh vesicles, together with ulceration in exposed areas, were present in 6 patients; onychia was noted in 2. Hepatomegaly was present in all but one case. Urine was collected in specially cleaned iron-free containers before and at various intervals during and after cessation of therapy. Chloroquine (300 mg base) was administered by one of us (J.J.F.T.) as tablets of chloroquine sulphate twice weekly. Patients remained in hospital for the first few weeks of treatment and were subsequently followed up as out-patients. They were closely watched for evidence of toxicity, and venous blood samples were withdrawn at intervals for liver function tests, including transaminases. In 4 patients the desferrioxamine test was performed before, during and after completion of chloroquine therapy according to Lundvall and Weinfeld (1969).

Animal Experiments

An experimental porphyria, closely resembling human symptomatic porphyria from the biochemical viewpoint, was induced in female Wistar rats weighing 150–200 g by a combination of iron overload and hexachlorobenzene (HCB) feeding (Taljaard *et al.*, 1972). After 60 days on hexachlorobenzene, when porphyrin storage is diffuse throughout the liver, HCB administration was stopped. Chloroquine was then administered by intraperitoneal injection to a group of these porphyric animals in repeated doses of 28 and 56 mg per kg body weight over a period of 3 weeks, as shown in Table V, and 24-hour urine collections were made while the animals were housed in metabolic cages. A comparable group of porphyric rats received intraperitoneal injections of physiological saline in equivalent volume on the same days as the first group received chloroquine, and their urines were also collected and stored at −20°C in the dark, pending determination of uroporphyrin.

In a second experiment (Table VI) the effect of a larger intraperitoneal dose of chloroquine (100 mg/kg) and the oral administration of carbon tetrachloride (0·1 ml/100 g in liquid paraffin) on the urinary and liver porphyrins was investigated in 2 further groups of rats, rendered porphyric as described above. These substances were administered on the second day after commencement of urine collections. The animals were sacrificed after 6 days, and their livers were removed and stored at −20°C in the dark prior to porphyrin determination.

Analytical Procedures

Porphyrins and porphyrin precursors were determined in urine and stools by well-known methods cited previously (Lamont *et al.*, 1961). Liver porphyrins were estimated by the method of Dresel and Falk (1956).

Urinary and liver iron estimations were performed as described by Peters *et al.* (1956), Lundvall and Weinfeld (1967) and Torrance and Bothwell (1968) respectively.

RESULTS

Patient Study

Initial biochemical data on the patients included in the present study are shown in Table 1.

Following commencement of chloroquine administration, all patients excreted increased amounts of porphyrin in the urine (Table II), reaching a peak in 3 to 28 days. Thereafter urinary uroporphyrin excretion declined over a period of months to levels which, although still abnormal, were well below those observed before treatment (Table II). With the exception of one patient (M.M.), who relapsed,

TABLE I.—*Initial Clinical and Biochemical Data on Porphyric Patients Studied*

			Clinical features*				Urine			Stool	
Patient	Age	Occupation	Vesicles and ulcers	Hyper-trichosis	Onychia	Hepato-megaly	ALA† (μg/ml)	PBG† (μg/ml)	URO† (μg/l)	COPRO† (μg/g dry wt)	PROTO† (μg/g dry wt)
S.M.	56	Road-building labourer	Face, scalp, chest, hands	+	Fingers	—	0·5	<1	3996	78	15
W.G.	51	Labour in eng. works	—	+	—	+	1·9	<1	1552	—	—
G.M.	52	Tractor driver	—	+	—	+	1·7	<1	556	—	—
M.M.	50	Labourer in eng. works	Hands	+	—	+	2·0	<1	3698	39	15
J.N.	38	Building labourer	Face, neck, hands	+	Fingers	+	3·6	<1	1564	116	63
J.K.	38	—	Hands, forearms	+	—	+	1·8	0	1141	24	65
E.S.	40	Building labourer	Face, neck, forearms, hands	+	—	+	1·7	<1	3386	36	16
A.K.	60	Farmer	Forehead, face, forearms, hands, lower legs	—	—	+	5·3	1·9	3988	146	73

* Hyperpigmentation and scarring of exposed areas present in all cases.

† ALA—δ-aminolaevulinic acid; PBG—porphobilinogen; URO—uroporphyrin; COPRO—coproporphyrin; PROTO—protoporphyrin.

TABLE II.—*Urinary Porphyrin and Iron Excretion in Porphyric Patients on Low Dose Chloroquine Therapy*

		Uroporphyrin (μg/l) and iron* (μg/l)										
			Weeks after commencement of chloroquine									
Patient	Duration of therapy (weeks)	Before chloroquine	1	2	4	6	8	10	12	16	20	> 24
S.M.	16	3996	—	5913	1801	1382	1303	469	—	344	116	415
		308	—	*593*	*285*	*507*	*192*	*296*	—	*125*	*152*	—
W.G.	11	1552	1245	1650	3042	2743	390	527	573	410	—	—
		96	*50*	*283*	*468*	*517*	—	*198*	*186*	*246*	—	—
G.M.	12	556	490	307	975	361	137	91	62	50	62	—
		75	—	*95*	*212*	*148*	*50*	*198*	*47*	*155*	—	—
M.M.	9	3698	12498	3527	2415	1461	639	3618	1879	—	—	12450
		—	*271*	*357*	*75*	*24*	*54*	*394*	*251*	—	—	—
J.N.	16	1564	1685	2859	339	91	83	91	162	120	—	—
		391	*278*	*695*	*126*	*25*	*152*	*26*	*146*	*116*	—	—
J.K.	2	1141	4976	33366	722	419	307	764	—	423	—	228
		403	*190*	*1247*	*12*	*25*	*308*	*308*	—	*424*	—	*139*
E.S.	4	3386	7540	3263	3208	—	—	—	—	—	2328	963
		—	*864*	*464*	*285*	—	—	—	—	—	*530*	*23*
A.K.	13	3988	8665	8619	4216	1905	1942	564	419	427	—	108
		—	*442*	*421*	*274*	*148*	*200*	*229*	*104*	*230*	—	*150*

* Values in italics.

biochemical improvement was maintained during the period of follow-up, which was usually limited by the patient's own decision to discontinue attendance as an out-patient. Five patients (G.M., W.G., S.M., J.N., M.M.) were observed regularly for 4 months on average. In 2 others (J.K., E.S.) the follow-up extended to 8 months; and, in one instance (A.K.), to a year.

In 7 patients the chloroquine-induced porphyrinuria was not accompanied by any signs or symptoms of the acute reaction which characteristically follows chloroquine administration in higher dosage in PCT (Sweeney *et al.*, 1965). However, one subject (J.K.) became pyrexial and complained of sweating, headache, malaise and pain in the right hypochondrium, whereupon administration of chloroquine was immediately discontinued. This reaction coincided with a massive increase in urinary porphyrin output (Table II) and moderate elevation of serum glutamic-oxaloacetic transaminase (SGOT) activity (Table III). The patient made a rapid recovery following this episode. Along with the biochemical amelioration, marked clinical improvement was noted in all patients. This included healing of existing lesions, decreased photosensitivity and a sense of "feeling much better". It is noteworthy that, despite advice to the contrary most of our patients did not abstain from alcohol during therapy. This was freely admitted on questioning, and on several occasions patients seen at follow-up were obviously under the influence of alcohol.

TABLE III.—*Serum Transaminase (GOT) Levels* During Chloroquine Therapy in Patients with Symptomatic Porphyria*

Patient	Before chloroquine	On chloroquine (at peak porphyrin excretion)	After chloroquine
S.M.	55	74	48
W.G.	73	48	105
G.M.	66	65	52
M.M.	7	106	90
J.N.	74	65	59
J.K.	62	198	48
E.S.	—	93	76
A.K.	68	80	67

* Karmen units.

Apart from the patient who developed an acute reaction to chloroquine, there was a slight increase in SGOT over pretreatment levels in 3 patients, while 3 others showed no increase (Table III).

Urinary iron excretion is displayed, together with uroporphyrin figures, in Table II. Before administration of chloroquine the mean urinary iron excretion was 255 $\mu g/l$ in 6 patients; in 2 patients (E.S., A.K.) this measurement was unfortunately not possible. This is consistent with the known urinary iron loss in siderotic patients (Moore, 1964). During chloroquine treatment the pattern of iron excretion resembled that shown for uroporphyrin, i.e. rising to a peak in the early phase and then falling off to lower levels.

Following intramuscular injection of desferrioxamine, markedly elevated urinary iron excretion was found (Table IV) in 4 patients tested. The desferrioxamine-chelatable iron rose from a mean value of 3·0 mg, compared with an upper limit of 2 mg in normal subjects (Dreyfus, 1964), to 8·1 mg during chloroquine therapy and then dropped to 4·8 mg after discontinuation of the drug.

TABLE IV.—*Results of Serial Desferrioxamine in Patients with Symptomatic Porphyria on Chloroquine Therapy*

Patient	Urinary iron (μg/24 hours) following desferrioxamine i.m.		
	Before chloroquine	On chloroquine	After chloroquine
S.M.	1440	4959	1732
W.G.	3630	10626	7400
G.M.	2587	9752	5227
M.M.	4400	6901	4950

Animal Experiment

Table V shows the effect of chloroquine administration on urinary uroporphyrin excretion in rats previously made porphyric by parental iron overload and 60 days of hexachlorobenzene feeding. From the results obtained in the control porphyric rats which were not given chloroquine it is apparent that uroporphyrin excretion in these animals may vary considerably. Hence the fluctuations observed in the chloroquine-treated group cannot be attributed to the drug. No reaction comparable to either the clinical or biochemical reaction seen in humans with PCT was observed in these animals during the 4 weeks of the experiment. A single larger dose of 100 mg/kg was equally ineffectual in this respect (Table VI), but the animals showed signs of toxicity, and one subsequently died. By contrast, when carbon tetrachloride was administered to porphyric rats, all manifested a marked increase in uroporphyrin excretion (Table VI), and their livers were subsequently

TABLE V.—*Effect of Chloroquine Administration on Urinary Uroporphyrin Excretion in Porphyric Rats*

	Urinary uroporphyrin (μg/rat/24 hours)			
	Chloroquine*		Saline†	
Day	Mean‡	S.D.	Mean‡	S.D.
1	433	51	434	112
2	455	95	450	82
3	345	66	291	73
6	201	30	147	28
7	182	56	199	39
8	271	39	291	48
9	272	73	238	31
10	334	70	301	94
11	286	174	323	63
13	299	58	254	103
15	339	63	285	68
17	323	59	313	132
19	412	56	369	68
21	225	134	309	82
23	246	11	269	66
25	370	16	302	59
27	164	39	299	83
29	125	37	266	56

* Chloroquine dosage i.p.: 56mg/kg on days 6, 24, 27; 28 mg/kg on days 10, 12, 15, 17, 19, 21.

† Saline dosage i.p.: equivalent volume to chloroquine solution on same days.

‡ Six animals in each group.

found to contain on average only about 12% of the porphyrin content of the chloroquine-treated animals.

TABLE VI.—*Comparison of Effect of Chloroquine and Carbon Tetrachloride on Urinary and Liver Porphyrins in Porphyric Rats*

Treatment	Rat no.	Urinary uroporphyrin (µg/rat/day)							Total liver porphyrins (µg/g dry wt)
		Day							
		0	1	2	3	4	5	6	
Chloroquine* 100 mg/kg	1	108	176	—	90	166	173	257	532
	2	172	207	235	268	117	119	123	433
	3	178	201	373	Died	—	—	—	—
Carbon tetrachloride† 0·1 ml/100 g	4	122	200	708	939	427	133	63	88
	5	77	83	221	514	273	69	—	36
	6	114	190	423	818	184	53	64	92
	7	74	77	115	561	146	61	—	35

* Chloroquine administered i.p. on day 2.

† Carbon tetrachloride administered orally in liquid paraffin (1 in 5) on day 2.

DISCUSSION

In recent years various forms of therapy have been advocated for patients with PCT. These include phlebotomy (Epstein and Redeker, 1968) chloroquine (Saltzer *et al.*, 1968) and chelating agents such as desferrioxamine (Wohler, 1964) sodium calcium edetate (Donald *et al.*, 1970) and penicillamine (Hunter and Donald, 1970a).

Phlebotomy has been shown to be most effective in producing lasting remissions (Epstein and Redeker, 1968), but is not always as convenient a mode of treatment as drug therapy. The majority of our African patients are reluctant to submit to repeated phlebotomies. Hence the quest for a safe, effective agent has continued. Chloroquine employed in dosage of the order of 0·5 to 1·0 g daily was found to be highly effective in reducing liver stores of porphyrin in PCT and promoting clinical improvement (Felsher and Redeker, 1966); but this regimen was not generally acceptable in view of the accompanying acute reaction, which in some patients can be alarming (Bloom, 1969).

On the other hand, preliminary experience with small, less frequent doses of chloroquine has been most encouraging (Saltzer *et al.*, 1968; Hunter and Donald, 1970b). The results obtained in the present study confirm that a low dose regime (0·3 g chloroquine base twice weekly) extending over several months is efficacious treatment in the majority of instances, both from the clinical and biochemical viewpoint. One patient, however, manifested an acute reaction even at this dose level, demonstrating the need for continuous close observation during treatment. Conceivably, still smaller doses might obviate all untoward reactions, yet prove therapeutically effective. In the case of the patient who relapsed (M.M.) it may be that treatment was discontinued too soon. On the other hand, patients J.K. and E.S. did not relapse during the period of observation (8 months), despite much shorter courses of treatment. With regard to subsequent therapy, M.M. should ideally undergo phlebotomy. If this is not feasible for the above-mentioned reason, the question arises as to whether a subsequent course of chloroquine is justifiable.

The mechanism whereby chloroquine acts in PCT is largely unknown. Early on it was suggested, on the basis of liver tenderness and raised serum transaminase activity accompanying the enhanced porphyrinuria, that the liver cell in this disease is particularly sensitive to damage by chloroquine (Sweeney *et al.*, 1962). Sweeney *et al.* (1965) compared this phenomenon to the primaquin sensitivity of glucose-6-phosphate dehydrogenase-deficient erythrocytes.

Certainly, damage to liver cells and even zonal necrosis can occur as a result of chloroquine administration in PCT (Felsher and Redeker, 1966), but whether this is a prerequisite for release of stored porphyrin by the liver is uncertain. In the present study 3 patients showed no rise in SGOT, despite good biochemical response to treatment. It may be, of course, that the slight damage which is not reflected in increased serum transaminase levels is in fact sufficient to allow escape of stored porphyrin. Alternatively, chloroquine may in some other way affect the ability of the liver cell to store porphyrin, e.g. by an effect of protein-binding of these pigments.

Scholnick and Marver (1968) reported that chloroquine forms with porphyrins a water-soluble complex which they suggested damages subcellular particles within the liver cell, with release of porphyrins from the liver. The findings in the animal experiments in the present study do not support this explanation for the effect of chloroquine. At the time when chloroquine administration began, the livers of these animals contained diffuse stores of porphyrin very similar in composition to that found in the livers of patients with PCT (Taljaard *et al.*, 1972). This would seem to be an ideal experimental situation for observing the effect of chloroquine on the porphyric liver; yet repeated administration of chloroquine (up to 100 mg/kg body weight) had no significant effect on liver stores or urinary porphyrin excretion in these animals. By contrast, the use of a known hepatotoxic agent, viz. carbon tetrachloride, clearly demonstrated that massive porphyrinuria follows damage to porphyrin-storing liver cells. These experiments, therefore, do not support the hypothesis that a necrotizing chloroquine–porphyrin complex is responsible for the porphyrinuria following chloroquine administration in PCT. On the other hand, it may be that the effective complex is formed between a metabolite of chloroquine and porphyrin, and possibly the porphyric rat liver is unable to metabolize the drug in the same manner as the porphyric human liver. Further studies are required to explain these phenomena.

Another question which remains unanswered is what effect of chloroquine underlies the lengthy remissions following its use in patients with PCT. If one accepts that liver cell damage and necrosis follow chloroquine treatment in these patients, it is logical to postulate that porphyric liver cells are removed and are replaced by healthy ones which do not produce excessive porphyrin. If, on the other hand, liver cell necrosis is not an essential part of the process, as was suggested previously, the above explanation would be invalid. The data on iron excretion obtained in the present study suggest an alternative explanation. It is well recognized that hepatic iron overload seems to be important in the pathogenesis of PCT (Lamont *et al.*, 1961; Uys and Eales, 1963), and the beneficial effect of phlebotomy has been attributed to a reduction in liver iron stores (Kalivas *et al.*, 1969). It has been pointed out, however, that the total amount of iron which must be removed to effect improvement is relatively small by comparison with the total liver iron in siderotic subjects (Haskins *et al.*, 1952). This suggests that the important iron pool in the liver with regard to PCT is small, separate from the main pool and preferentially mobilized by phlebotomy. In the patients in the present study urinary iron excretion increased simultaneously with the increase in porphyrin output during chloroquine therapy. Also, the desferrioxamine-chelatable iron increased at this time, possibly indicating a greater mobility of a specific liver iron pool. It could be, therefore, that one of the results of chloroquine administration in PCT is the depletion of a particular fraction of the liver iron stores which is essential for the continued overproduction and accumulation in the liver of higher carboxylated porphyrins.

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INTERMITTENT CHEMOTHERAPY IN THE TREATMENT OF TUBERCULOSIS CUTIS

A PRELIMINARY REPORT

V. S. RAJAN AND Y. S. GOH

Summary.—Seven cases of tuberculosis verrucosa cutis were treated by the intermittent (twice weekly) fully supervised regimen of streptomycin and high dosage isoniazid. Responses were very impressive—6 months of treatment produced complete healing, leaving residual scars.

In 1964 an intermittent regimen of high dosage isoniazid, plus streptomycin given twice-weekly under supervision, was shown to be as effective in the treatment of pulmonary tuberculosis as a standard unsupervised daily regimen of isoniazid plus PAS (Tuberculosis Chemotherapy Centre, 1964). Subsequent reports from the same centre and elsewhere (Nazareth *et al.*, 1966; Menon, 1968; Devi, 1972) have confirmed the efficacy of such a regimen both in the attainment of bacteriologically quiescent disease at one year and also in its maintenance over a follow-up period of 4 years. Theoretically, this method of treatment should be equally successful in other forms of tuberculosis.

We report here our experience in the treatment of tuberculosis cutis, using an intermittent regimen of twice-weekly streptomycin and high dosage isoniazid.

PATIENTS AND METHODS

From late 1970, 7 cases of tuberculosis of the skin seen in our hospital were treated by the intermittent fully supervised regimen as follows: (1) streptomycin 1 g intramuscularly plus (2) isoniazid 14 mg/kg body weight in a single dose.

Patients called at the hospital on Mondays and Fridays and were given the injections, which were followed immediately by the single dose of isoniazid under direct supervision. Pyridoxine was also given at the same time to offset possible neurological side effects due to high dosage isoniazid.

The diagnosis of the cases was made on clinical features and confirmed by histological study of biopsy tissue. No routine culture of the biopsy for mycobacteria was done because past experience had shown a poor yield. Syphilitic gumma was excluded by a negative VDRL in all cases.

Morphologically all cases in this study were of the verrucous type (tuberculosis verrucosa cutis). No case of classical lupus vulgaris was seen. This infrequency of lupus vulgaris is not unusual in our predominantly Chinese population (Wong *et al.*, 1968).

CLINICAL FEATURES

Table I shows the clinical features, the response to treatment and the follow-up.

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TABLE I

Case no.	Name	Age and Sex	Site	Size (cm)	Duration of lesion (years)	Chest x-ray	Mantoux test (1 TU) (mm)	Duration of treatment (month)	Length of follow-up (months)	Present status
1	T.G.L.	16M	(R) Heel	5	1	NAD	N.D.	10	11	Scar
2	W.A.K.	23M	(L) Elbow	4	7 mth.	NAD	8	8	14	Scar
3	T.P.Y.	45M	(R) Fourth finger dorsum	2	6 mth.	NAD	10	2	On treatment	Lesion flat
4	N.K.L.	23M	(R) Foot sole	5	10	NAD	20	6	12	Scar
5	C.L.Y.	16M	(R) Groin	12	4	NAD	Neg.	6	7	Scar
6	C.Y.W.	18M	(R) Knee	5	7	NAD	10	3	On treatment	Scar
7	K.H.H.	34F	(L) Foot dorsum	4	10	NAD	N.D.	6	10	Scar

RESULTS

The response was very impressive; regression was noted as early as the third week of treatment; by the sixth week flattening of the lesions was obvious. By the third month all lesions were flat; and, by the sixth month, only thin atrophic scars were seen, with no signs of activity.

Side effects.—None was noted.

Termination of treatment.—In view of the very impressive response, it was decided to terminate treatment at 6 months. Before the adoption of this policy, 2 early cases (Cases 1 and 2) had treatment up to 10 and 8 months respectively, and both had shown healing by 6 months.

Follow-up.—The longest period of follow-up was in Cases 2 and 4—6 months after cessation of therapy. No relapse was noted. Follow-up of the other cases is still in progress.

DISCUSSION

In warty tuberculosis and lupus vulgaris the lesion is usually confined to the skin, the immunity is high and the bacilli are scanty and may be attenuated. Thus a greater latitude of treatment is permitted with this disease (Wilkinson, 1968).

Bruck (1964) treated 167 cases of lupus vulgaris with isoniazid alone, in standard doses, for a year, obtaining a 93·7% cure rate. Forstrom (1969) has shown that isoniazid alone is sufficient to produce cure, although it must be given for up to 18 months to prevent relapses. Others give combined therapy, especially if there is a possibility of other organ involvement (Coskey, 1967). Wehnert (1950) obtained a 97·5% cure rate using PAS/isoniazid.

In Singapore, tuberculosis, although declining in incidence, is still an appreciable health problem and, of 21 cases of tuberculosis cutis seen over a 2-year period (Goh and Rajan, 1972) 2 had active pulmonary lesions. Hence in this series we have elected to use combined therapy even although all the cases had normal chest x-rays.

To date, all regimens used in the treatment of tuberculosis cutis are unsupervised, and the patient must be relied upon to take his drugs daily. Even with excellent regimens, it is the irregular or negligent patient who relapses (Fox, 1968; Forstrom, 1969).

The intermittent regimen overcomes this difficulty as it is entirely supervised. The level of therapeutic effectiveness has been shown to be equal to that of the best daily regimens and, although individual doses of the drugs are higher, it has lower chronic toxicity because the total weekly dosage is less (Fox, 1968).

These advantages are well borne out in this series. There were no defaulters, and no side effects were noted. As healing had taken place by 6 months, we feel that this period of treatment is adequate. It is of course too early to conclude if this policy is wise, and we are following the patients closely to detect any relapse.

Studies in Madras (Tuberculosis Chemotherapy Centre, 1960) have shown that isoniazid need not be given continuously to achieve its therapeutic effect. This opens the interesting possibility that, in countries where isoniazid can be used alone, a trial of intermittent high dosage isoniazid can be carried out in tuberculosis cutis.

We are indebted to Dr Chew Chin Hin, Consultant Chest Physician, Tan Tock Seng Hospital, for his stimulating thoughts on the chemotherapy of tuberculosis.

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Case Report

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LOCALIZED ACNEIFORM ERUPTION FOLLOWING COBALT IRRADIATION

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Summary.—A woman aged 45 developed a severe dense eruption of open and closed comedones and follicular cysts, confined to the irradiated sites, 5 months following deep cobalt teletherapy for a mediastinal lesion. Histological study revealed typical open and closed comedones, ruptured follicular cysts with surrounding foreign body reaction, and features of steatocystoma. It is postulated that the severe acneiform eruption reflects the interaction of multiple acneigenic factors in which cobalt irradition served to localize the response.

SUPERVOLTAGE cobalt 60 teletherapy is being widely used in the treatment of deep-seated malignancies. Despite the skin-sparing advantages of cobalt irradiation, skin reactions have been observed in about a quarter of the patients so treated (Liegner and Michaud, 1961). This report extends the spectrum of adverse local eruptions following supervoltage irradiation to include the case of a woman aged 45 who developed a severe eruption of open and closed comedones and follicular cysts, confined to the irradiated sites, 5 months following cobalt therapy for a mediastinal lesion.

CASE REPORT

A white woman aged 45 was seen in consultation at the Hospital of the University of Pennsylvania in April 1971 for evaluation of a progressive " radiation dermatitis ". She had been admitted to the hospital for further investigation of a right-sided pulmonary mass considered to be carcinoma of the lung.

A superior venacavagram performed at another hospital in April 1970 showed partial occlusion of the superior vena cava by an extrinsic tumour.

The patient then received a course of systemic nitrogen mustard therapy without subsequent improvement. One month later, in August 1970, Cobalt 60 teletherapy was instituted. The treatment schedule consisted of 15 treatments over a 3-week period. A total tumour dose of 4000 rad was administered, with resultant shrinkage of the mediastinal mass and improvement in the superior vena cava syndrome. The anterior chest wall portal received 2200 rad total dosage delivered to the tumour, while the posterior chest wall portal received a total of 1800 rad.

In September 1970 a thoracotomy was performed and biopsy of the tumour revealed non-specific inflammation and fibrosis. Isoniazid, ethambutal and prednisone therapy were then administered and continued until January 1971. The prednisone, initially maintained at a dosage of 40 mg daily for one month, was then gradually tapered and stopped.

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Following the cobalt radiation therapy in August, the patient noticed no erythema; but, from September 1970 until February 1971, she noticed slowly increasing roughness and pigmentation of the irradiated fields on her chest and back. In mid-February 1971, about 5 months following the last cobalt treatment, these sites became progressively indurated, painful and pruritic. Her hospital course was complicated by cryptococcal pneumonia, for which she received a one-month course of intravenously administered amphotericin B.

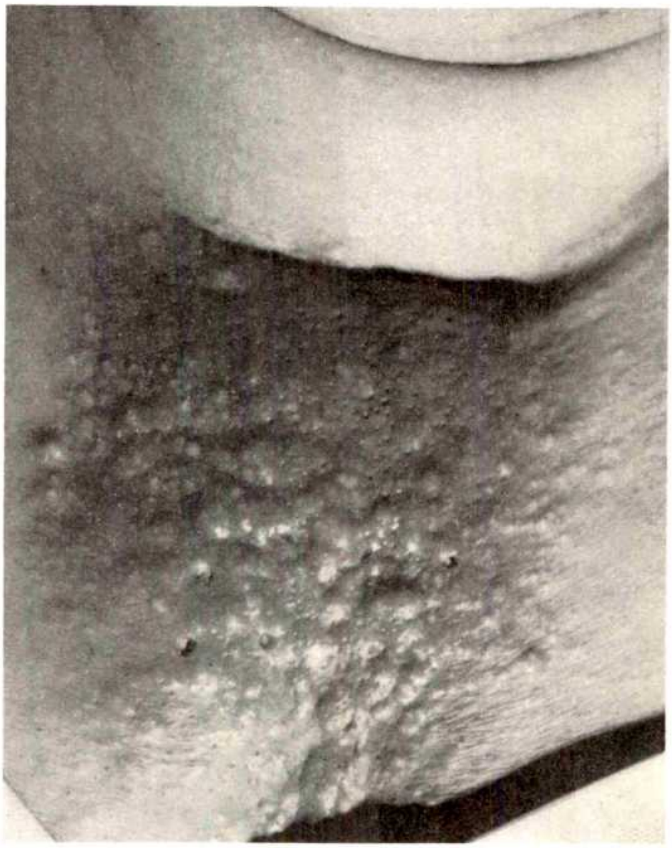

FIG. 1.—The skin overlying the sternum was erythematous, thickened, indurated, and contained numerous large cysts.

Past history revealed she had experienced acne as a teenager of sufficient severity to leave scars on her face, chest and back.

The patient presented as an obese white woman with mild Cushingoid facies. On the upper chest overlying the sternum was an 11 × 12 cm rectangular area which was erythematous, thickened, indurated and tender (Fig. 1). The area was studded with numerous dilated pilosebaceous orifices, closed and open comedones, and follicular cysts from which keratinous debris could be expressed (Fig. 2). Similar changes, but of a lesser degree, were localized to a 11 × 12 cm rectangular area of the upper mid-back. Occasional open comedones were noted on her forehead, neck and back. Some pitting and atrophic scarring were present on the face, chest and back.

Laboratory Findings

Admission chest x-ray on February 19th, 1971, showed a large mass on the right side of the chest with mediastinal involvement. A biopsy of the superior mediastinal mass on March 8th revealed caseating granulomata. A histoplasmin skin test was positive. Special stains and cultures for acid-fast bacilli, fungi and bacteria were negative. Pulmonary function studies indicated severe restrictive lung disease.

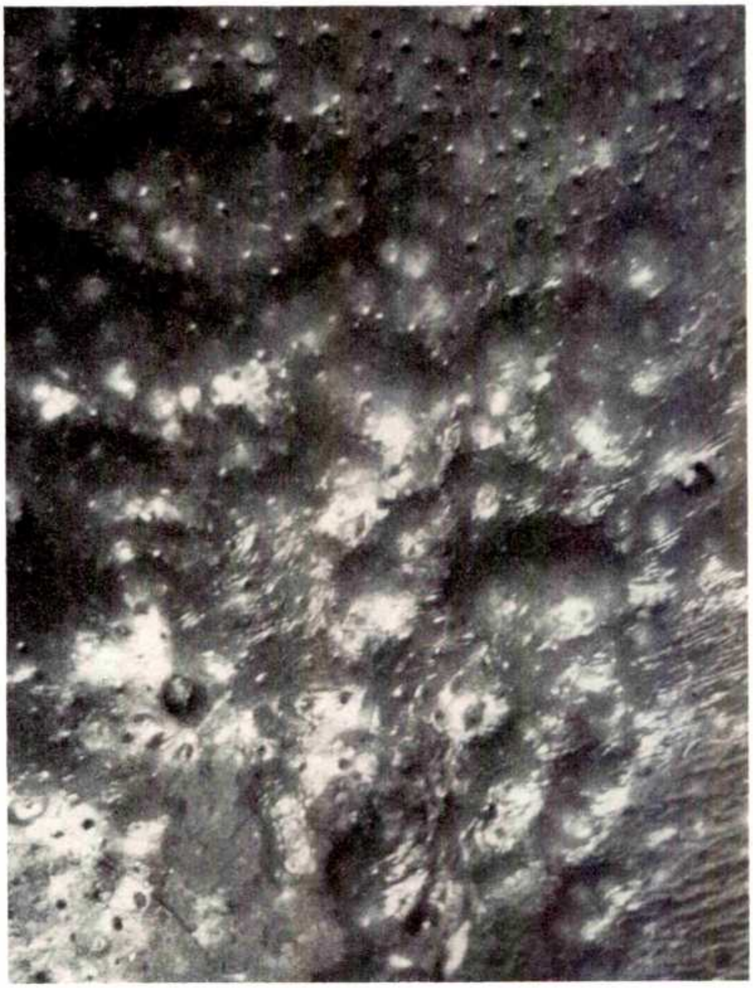

FIG. 2.—Close-up view of the skin of the anterior chest, showing numerous dilated follicular orifices, closed and open comedones, and follicular cysts.

Cultures of numerous cysts and comedones revealed scanty growth of *Corynebacterium acnes*. Cultures for fungi were negative. Several skin biopsies showed typical open and closed comedones (Fig. 3), ruptured follicular cysts with surrounding foreign body reaction (Fig. 4). Scattered minute sebaceous glands were noted in all specimens. In addition, one cyst showed the features of steatocystoma (Fig. 5). The dermis was thickened, oedematous, and showed area of fibrosis. The subcutaneous tissue appeared normal.

The patient experienced severe pain on attempts manually to extract the comedones and cystic lesions, even after prior sedation with intravenously administered Valium.

Retinoic acid 0·05% in propylene glycol-ethyl alcohol solution was applied topically once daily to the involved sites. Although many open comedones were extruded, leaving observable patulent pilosebaceous orifices, the follicular cysts resolved more slowly, as did the symptoms of pain and burning, and the alarming appearance was only slightly improved after 4 months of therapy.

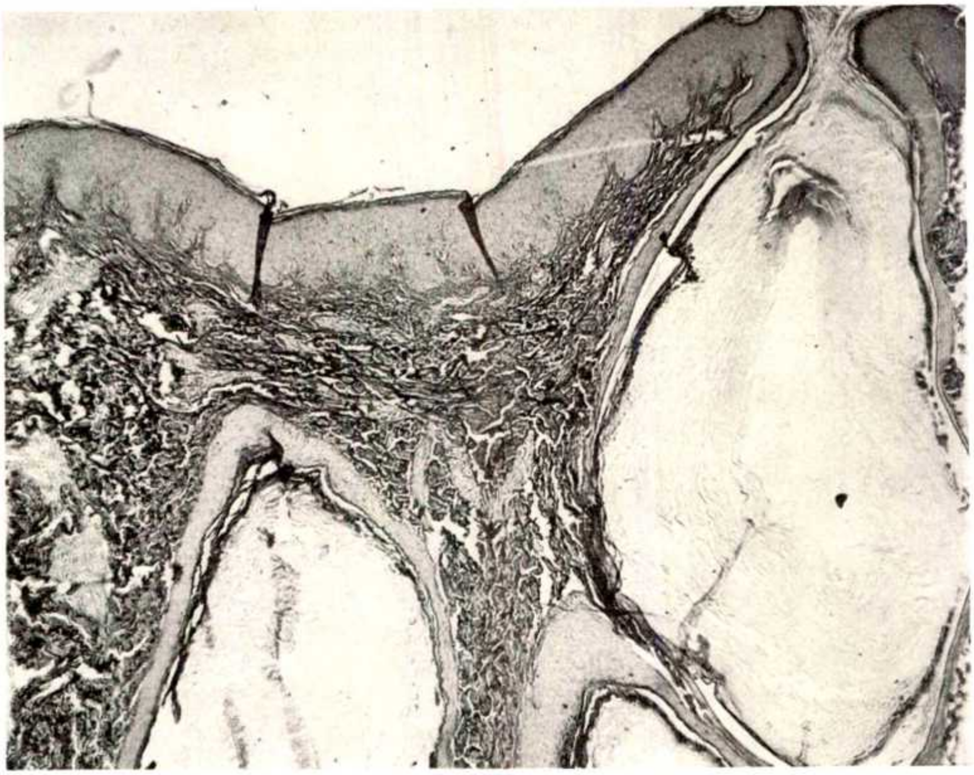

FIG. 3.—Photomicrograph (chest): open and closed comedones are present. The elastic tissue appears normal. (Verhoeff × 50)

DISCUSSION

Although acneiform eruptions can be produced by exposure to a wide variety of external agents, radiation-induced changes of this type are less common, and have been reported as mild eruptions of comedones by several authors. Bluefarb (1947) first called attention to a ring of comedones around the scar of facial epithelioma treated with Roentgen radiation (2160 rad once a week for 3 weeks). Ronchese (1950) observed the same phenomenon in 3 cases of basal cell epithelioma (3600 rad filtered and given in 3 exposures). Swift (1956) reported localized comedones and pustules following deep x-ray therapy for breast cancer (2000 rad) in a woman also receiving systemic testosterone therapy, but did not specify the x-ray source. No biopsy material was obtained.

Acneiform eruptions have not been recorded following deep cobalt teletherapy. To our knowledge, cobalt irradiation is given because of its skin sparing effect, but erythema, pigmentation and dry and moist desquamation are commonly observed during therapy in the immediate post-treatment period. Delayed reactions are more unusual and consist of the insidious development of subcutaneous fibrosis from 2 months to 4 years after treatment, when large field sizes and large amounts of radiation are employed (Liegner and Michaud, 1961).

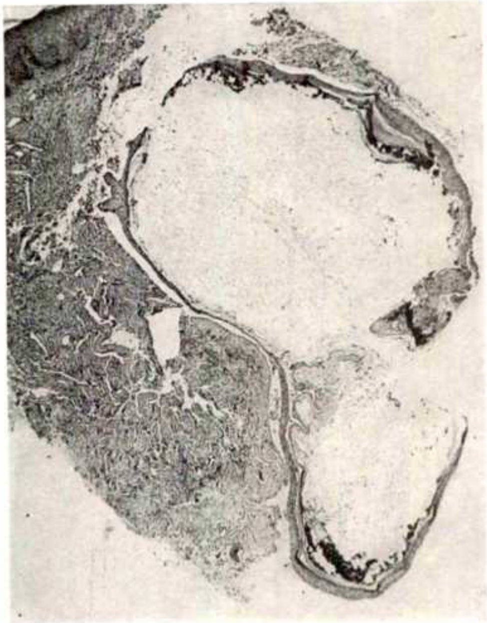

FIG. 4.—Photomicrograph (back): within the dermis is a large cyst lined with stratified squamous epithelium and containing keratin. Outside the wall of the cyst in one area is a dense infiltrate of lymphocytes, neutrophils, histiocytes and foreign body giant cells. (H. and E. × 40)

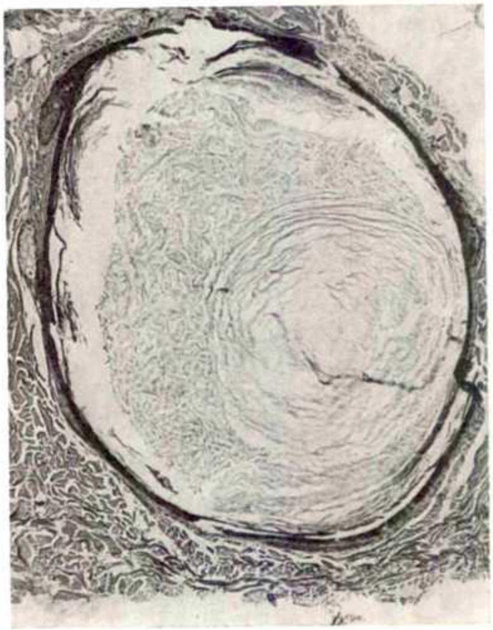

FIG. 5.—Photomicrograph (back): deep in the dermis is a large cyst lined with squamous epithelium and containing keratin. Sebaceous structures are present in or adjacent to the cyst wall. Note resemblance to steatocystoma multiplex. (H. and E. × 80)

Our patient developed a delayed localized acneiform eruption on the chest and back, limited to the irradiated areas. This was accompanied by a striking induration of the involved sites. Histological study revealed an array of findings, including open and closed comedones, ruptured follicular cysts with foreign body reaction, and features of steatocystoma. The dermis was thickened and fibrotic.

The cobalt 60 source emits gamma radiation which penetrates deep tumours with relative sparing of the more superficial tissues. The dose delivered rises with skin depth for the first few mm and then remains constant to a depth of 1 cm. Maximum ionization actually occurs at about 0·5 cm below the skin, and a small but significant amount is scattered in the overlying skin and subcutaneous tissue.

A fraction of the penetrating gamma radiation exits through the skin directly opposite the irradiated site (Braestrup, 1959).

Substantial amounts of radiation were absorbed by the skin of the patient. Significantly, about 50% of this total dosage was delivered in the first 5 days of treatment. The skin of the anterior chest wall (0·5 cm) received a total of 5056 rad, and that of the back (0·5 cm) 4484 rad.

The severe acneiform eruption most likely reflected the interaction of multiple factors in which cobalt irradiation served to localize the response. The patient had a past history of acne vulgaris and a recent 5-month course of systemic steroids and isoniazid therapy. Systemic corticosteroids are known to produce follicular hyperkeratosis (Frank, 1971) and sebaceous gland hypertrophy (Ebling and Rook, 1968), and may have served to prime the pilosebaceous structures already genetically predisposed to acneigenesis. Isoniazid can also produce acneiform eruptions, but comedones are usually absent, and this drug may not have contributed to the final efflorescence of the follicular lesions in this case. We postulate that the superimposed gamma radiation initiated chronic follicular inflammation and augmented follicular hyperkeratosis resulting in localized follicular cyst and comedone formation. This view is supported by Strauss and Kligman (1959) who demonstrated that sustained follicular inflammation of diverse causes stimulated follicular hyperkeratosis.

A striking clinical, and perhaps analogous, example may be the grouped comedones seen in nodular elastoidosis of Favre and Racouchot, where prolonged exposure to sunlight is the essential factor leading to the follicular hyperkeratosis and comedone formation.

In summary, this report calls attention to a resistant acneiform eruption as an adverse skin side effect of deep cobalt teletherapy.

Dr Margaret Gray Wood interpreted the histopathological sections. Mr Edward Glifort prepared the photographs.

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Comment

THE NATURE, PATHOGENESIS AND STAINING PROPERTIES OF AMYLOID

Amyloid can be defined as a predominantly extra-cellular deposition of a protein complex which has certain structural, histochemical and optical properties. In recent years there has been a considerable amount of research into amyloidosis. Part of the current interest is the peculiar liability for amyloid degeneration to occur in ageing tissues, both in humans and in various animal species. This paper summarizes recent developments in amyloid research, with special reference to the structure and composition, pathogenesis and staining properties of amyloid.

Structure and Composition

It is now known that all types of amyloid deposits have a characteristic fibrillary ultrastructure (Cohen, 1965). Analyses of human amyloid tissue have revealed 2 specific components: (1) Filaments, measuring 7·5 Å, composed of a series of about 5 subunits termed protofibrils (Shirahama and Cohen, 1967); (2) Plasma or P-components, measuring 100 Å, which consist of stacked pentagonal structures surrounding a central core (Bladen *et al.*, 1966). These 2 components of amyloid can be distinguished on the basis of their immunological and staining properties (Cathcart *et al.*, 1970). X-ray diffraction studies have shown that amyloid has the same cross-β configuration as a number of other naturally occurring proteins (Eanes and Glenner, 1968; Bonar *et al.*, 1969). These components consist chemically of a series of protein-mucopolysaccharide complexes which do not appear to be chemically related to collagen. Specific analyses have shown that amyloid consists of approximately 85% protein and 15% carbohydrate Ashkenazi *et al.*, 1968), and is rich in the amino-acid tryptophan and mucopolysaccharides, particularly the heparan sulphate component (Bitter and Muir, 1966). However, amyloid from different individuals may vary in precise composition of proteins and amino-acids (Harada *et al.*, 1971). Black (1971) has suggested that the individual deposits in lichen amyloidosus may also vary in their composition. Immunofluorescent studies have shown the presence of γ-globulin (Vazquez and Dixon, 1956; Schultz *et al.*, 1966), fibrinogen and of β-1C complement (Lachmann *et al.*, 1962) in amyloid deposits. However, not all investigations (e.g. Calkins *et al.*, 1958) have been able to confirm the presence of γ-globulin in amyloid, and Cohen (1968) has suggested that a variety of compounds, including immunoglobulins, may be absorbed or become loosely bound to amyloid in a non-specific way.

Pathogenesis

Two main theories of the formation of amyloid exist: (1) that it arises by the precipitation of a serum component; (2) that it is secreted locally by cells.

I. *Precipitation of a serum component.*—Letterer (1934) suggested that amyloid was formed as a precipitate of antibody-antigen complexes, but this theory has never been confirmed. No one has demonstrated any quantitative or qualitative alterations in serum levels of immunoglobulins that could be correlated with amyloid formation (Giles and Calkins, 1958; Willerson *et al.*, 1969). Furthermore, it has been shown that immunoglobulins are not an integral component of amyloid (Cohen, 1965; Cohen and Paul, 1963). Recently, further interest in the precipitation theory of amyloid formation has been aroused by the studies of Glenner *et al.* (1971a), who have shown that, in some cases of amyloidosis, the major protein component of the amyloid fibrils are portions of light polypeptide chains. Glenner *et al.* (1971b) have produced amyloid fibrils from some human light chain components (Bence Jones proteins) by means of proteolytic digestion, and have suggested that this finding establishes a firm relationship between amyloidosis and immunoglobulins.

II *Local secretion by cells.*—Teilum (1956, 1964) has proposed that " the process of amyloid formation is dependent on a biphasically developed aberration of the protein-synthesizing function of mesenchymal (reticulo-endothelial) cells ". In the initial (pyroninophilic) phase there is a proliferation of reticulo-endothelial cells and a rise in the serum γ-globulin. The second or amyloid phase depends on a suppression of the proliferating reticulo-endothelial cells which become PAS-positive and then secrete amyloid. Ranløv (1968) has suggested that the link between these 2 phases could be the release of a " transfer factor " of nuclear nature from the pyroninophilic cells, which results in the reticulo-endothelial cells synthesizing amyloid. However, the suggestion made by Ranløv that amyloid formation depends solely on a transfer factor secreted from pyroninophilic cells has not been corroborated by the recent work of Hardt (1971). Cathcart *et al.* (1970) showed that, after repeated injections of casein, used during the experimental induction of amyloidosis in guinea-pigs, macrophage inhibition tests were no longer positive, whilst cellular immunity to other antigens remained intact. Cathcart *et al.* suggested that a state of immunological tolerance to injected casein had developed, and they postulated that tolerance to other specific antigens may play a central role in the pathogenesis of amyloidosis. Hardt and Claësson (1971) were unable to reproduce the findings of Cathcart *et al.* and found only non-specific impairment of the cellular immunological response. Hardt (1971) has suggested that pathogenetic mechanisms of amyloidosis include the co-operation between an amyloid-inducing factor (AIF), operating intercellularly, and the sustained presence of a specific antigen. This results in an alteration of macrophage function which then leads to the synthesis of amyloid. In primary localized cutaneous amyloidosis (lichen amyloidosus) electron microscopic studies have suggested that amyloid fibrils are secreted by fibroblasts in the papillary dermis (Hashimoto and Onn, 1971).

Staining Properties of Amyloid

It has long been recognized that the special stains employed in the detection of amyloid deposits may be unpredictable in their staining reactions, or lack the necessary specificity for positive identification. This state of affairs is particularly unfortunate for dermatohistopathologists, as a number of hyaline materials (e.g. lipoid proteinosis, colloid milium) other than amyloid may also be found in the dermis.

Three stains and techniques are mainly employed for the histological demonstration of amyloid; (1) Staining with triphenylmethane dyes (e.g. methyl violet) to demonstrate " metachromasia "; (2) the substantive cotton dyestains (Congo red, Sirius red), with or without fluorescence or polarization microscopy; (3) fluorescence with thiazole dyes (e.g. thioflavine T). In a recent evaluation of the various amyloid staining procedures, Cooper (1969) concluded that thioflavine-T fluorescence, Congo red fluorescence and Sirius red staining constituted efficient screening methods for detecting amyloid deposits, but that they lacked the specificity necessary for positive identification. However, Cooper found that amyloid deposits could be specifically identified by finding green birefringence with Congo red or Sirius red when the sections were examined using polarized light. Unfortunately, this method was often inadequate in detecting small deposits of amyloid. Romhányi (1971) has described a modification of the Congo red method, which he claims distinguishes the stronger birefringence of amyloid from the weakly birefringent collagen fibres. Romhányi found that, if the tissue sections were treated with proteolytic enzymes and then mounted in gum arabic, the general background staining decreased and this selectively increased the birefringence of amyloid. Romhányi also found that the enzymatic digestion of elastic fibres prior to the Congo red staining facilitated the exact localization of amyloid deposits within vessel walls.

Unfortunately, the staining properties of Congo red, Sirius red and crystal violet may vary considerably, particularly in formalin-fixed tissue. Wolman (1971) found that amyloid stained a red colour with toluidine blue when the sections were examined under polarized light. Wolman's results suggested that, although this colour change was specific for amyloid and did not appear to be affected by the method of fixation, it was not sensitive enough in detecting small deposits of amyloid. The reliable identification of small deposits of amyloid is of paramount importance to dermatologists, since in certain types of primary localized cutaneous amyloidosis (e.g. macular amyloidosis) the deposits of amyloid are often small and sparsely distributed in the papillary dermis. In 1965 Hashimoto *et al.* showed that the amyloid was more strongly stained with Congo red and methyl violet in frozen sections than paraffin-embedded material. Black and Wilson Jones (1971) confirmed this finding and found the method reliable for identifying the sparse amyloid deposits of macular amyloidosis. It is therefore suggested that, if cutaneous amyloidosis is suspected clinically, the biopsy should be frozen immediately, rather than being fixed in formalin, and that frozen sections be stained for amyloid with Congo red and methyl violet. It has been the experience of the author that the extent of the smaller amyloid deposits in the skin is often better demonstrated by thioflavine-T fluorescence than by any of the other special stains.

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Br. J. Derm. (1972) **87**, 284.

Obituaries

JOHN LEWIS FRANKLIN

One of the older school of clinical dermatologists, J. L. Franklin passed away suddenly on May 19th, 1972 at the age of 68. Although he had been in declining health for some years he continued his private practice up to the day of his death. He had retired from the N.H.S. three years earlier.

John as he was always affectionately known to his friends was educated at Epsom College and started his medical training at Pembroke College, Cambridge. He obtained a half-blue for Epée and maintained his interest in fencing most of his life. He proceeded to St George's Hospital for his clinical training and qualified M.R.C.S., L.R.C.P. in 1927. He graduated M.B., B. Chir. two years later, at a time when a thesis was still required for the M.B. His subject was the treatment of lupus erythematosus with gold and this reflected the main interest of his later life, namely the treatment of patients. In 1931 he passed the examination for the M.R.C.P.(London) and obtained the Cambridge M.D. in 1933. He was elected F.R.C.P. in 1955.

John was appointed to the staff of Westminster Hospital at a very early age as both dermatologist and venereologist. At various times he also held consultant appointments at Edenbridge Hospital, Princess Beatrice Hospital and the Seamen's Hospital, Greenwich. He was one of the last dermatologists on the staff of a London Teaching Hospital to give way to the venereologist. He did not consider that venereology should be a speciality in its own right. His comments that there was " no such animal " as a venereologist did not endear him to his colleagues in that speciality. He eventually handed over the department when the number of cases attending V.D. clinics began rising steeply after reaching a very low ebb in the post-war years. He never quite accepted that penicillin alone was the treatment of choice for syphilis, preferring to rely on the metals which had been well tried over so many years.

John was not greatly interested in research and he distrusted the full-time research worker. His publications therefore were few in number. With Dore he published a short and eminently readable text for students. Unfortunately all unsold copies and the blocks were destroyed in the blitz so that the work went out of print. John was a good and amusing teacher. He was a firm believer in the psychosomatic approach to dermatology and neurodermatatis was his favourite diagnosis. His Wednesday afternoon teaching clinic was well attended by students even though Wednesday afternoon was the traditional sports' time at Westminster. His successor recalls with envy the days when all students could spend 3 months in the dermatology department. The cluttered curriculum of the present day has put an end to this.

John was a man of crazes. In succession these were gardening, fishing, horse racing, flashy cars and the stock exchange. Except for the last these were short lived and soon forgotten. During the horse racing days he would bring a portable radio into the outpatient department on the first Wednesday of June each year to listen in to the broadcast of the Derby. The clinic restarted when the race finished. He believed in enjoying life to the full. He was also impulsive. The writer remembers well an occasion when John had offered to take him by car to the annual B.A.D. meeting in Oxford. We set off sedately in his Bentley until we were some 15 miles from Harley Street when he remembered something that had to be done in his room. We sped back to Harley Street and then back at full speed to the point we had previously reached when he relaxed and we continued at a leisurely pace once more.

John was a good mixer and a real character. At meetings he could always be picked out by the red carnation which he invariably wore. He was kind and generous and will be sadly missed by his many friends. He was married three times, the first two marriages ending in divorce. He had a son and daughter by his first wife. We extend our sympathy to his widow and family.

P. D. Samman

E. C. RITTER

Eric Camillo Ritter, who had retired from practice as consultant dermatologist in Lincoln, died on March 1st at the age of 63.

He was born on April 20th, 1908 and read medicine at the University of Glasgow where he graduated M.B. Ch.B. with commendation in 1930. After house appointments in Glasgow he went to the London Hospital for postgraduate study and became a Member of the Royal College of Physicians of London in 1934. He returned to Glasgow and worked in the dermatology department of the Victoria Infirmary under Dr Herbert Brown of whom he frequently spoke with affection. He became Assistant Physician for Diseases of the Skin and Senior Hospital Medical Officer at the Victoria Infirmary and V.D. Officer for the City of Glasgow.

In 1938 he moved to Lincoln in general practice and the following year was appointed consultant dermatologist to the Lincoln group of hospitals. Until a year or two after the beginning of the National Health Service he was the only dermatologist in Lincolnshire, the second largest county in England, and visited Grimsby, Scunthorpe and Boston with all the miles of travelling this entailed. At the time of his retirement he was conducting clinics in Boston and Grantham as well as Lincoln. There are thus many thousands of patients in Lincolnshire who remember him with gratitude and affection. His warm and friendly nature made him the sort of doctor to whom they could pour out their troubles and it is in keeping that most of his publications were on the psychosomatic aspects of skin disease.

Having worked for so many years without any day to day links with other dermatologists he was keenly aware of the importance of attending meetings to keep up with the latest advances in his subject. His distinguished and dapper figure with a mop of white hair, light grey suit and plain pastel-coloured tie was a familiar sight at meetings of the dermatological section of the Royal Society of Medicine, the North of England Dermatological Society and the British Association of Dermatology.

Eric came of a line of professional musicians from Graz in Austria. His father, who had been taken to Glasgow in childhood as it was at that time one of the cultural centres of Europe, became a professional violinist. With this background it is not surprising that music became a dominant interest in his life. In childhood, visiting relatives in Austria with his father, the music of the country inspired him to take up guitar playing and singing. He also played the piano and the flute at which he became remarkably skilful. One of his greatest pleasures was an intimate musical evening with his friends. Painting under the guidance of a local artist of repute, modelling in clay, and wide reading had made this quiet, modest and artistic man the most entertaining of companions who would converse on any subject, displaying a dry sense of humour which went well with a faint Scottish accent.

He retired at 60 because of ill health and his last years were clouded by disabling illness which he bore patiently, much sustained by the devotion of his wife. His colleagues, friends and patients miss him sadly. He is survived by his wife and two sons, one of whom is a medical student.

Ronald Church

GILBERT HENLY

Dr G. Henly, formerly consultant dermatologist to the Birmingham Skin Hospital, died at Budleigh Salterton, Devon, on November 24th, 1971. He was 71.

Gilbert Henly was born on January 8th, 1900 and educated at Birmingham University, where he graduated M.B., Ch.B. in 1922, taking the London Conjoint diploma the following year and proceeding M.D. in 1926. He was in general practice in Birmingham and Wolverhampton until 1937, and his early interest in dermatology and venereology led to his joining the staff of the former Midland Hospital, Birmingham, where he subsequently became honorary dermatologist from 1937 until the hospital closed after war damage. He was also on the honorary staff of the venereal diseases department of Birmingham General Hospital from 1939 to 1960. From 1941 until his premature retirement in 1962 he was consultant dermatologist to the Birmingham Skin Hospital.

Devoid of academic pretensions, Gilbert Henly was a true clinician with a wide practical knowledge of his specialities in general and of the medicolegal aspects of dermatology in particular. A kindly and gentle man, his sympathetic and human approach endeared him to his patients and colleagues both young and old. His shy, almost diffident manner concealed a shrewd judgement and a wealth of strongly held opinions on matters on which, from his clinical experience, he could speak with undeniable authority. Never enjoying the most robust health, he chose to retire at the age of 62 and went to live quietly at Exmouth. He had a strong artistic bent, and apart from golf and gardening he now found time to develop his talents as a pianist and latterly, when his activities were more restricted, turned to painting.

He is survived by his wife and a son, both of whom are doctors.

Stanley Wood

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G. H. RITTERMAN

Gedeon Heinrich Ritterman, known affectionately as " Ritty or Heini ", died as a result of an accident on January 20th, 1972, aged 68.

Born and educated in Vienna, he qualified M.D. in 1932 and soon decided to make dermatology his career. He was on the staff of the Rothschild Hospital.

After Hitler's invasion of Austria in 1938, Dr Ritterman emigrated to India and practised dermatology in Calcutta. He volunteered for Service with H.M. Forces, and was commissioned in 1942. He served mainly in India Eastern Command, attaining the rank of Major, and his services as Specialist Dermatologist in the R.A.M.C. were greatly appreciated.

He came to England in 1948 as Ex-service Registrar to St John's and was appointed Consultant to the Lister, North Hertfordshire and Stevenage Hospitals in 1952, retiring in 1968.

An accomplished linguist and an amusing raconteur, he was a most likeable cultured man of the world whose interests ranged from the arts, particularly literature and the theatre, to sociology and politics.

Essentially a practical dermatologist with a flair for therapy, he had immediate rapport with his patients from every walk of life, whom he saw in large numbers and at frequent intervals at every hospital session.

He was an unusual and charming character who will be sorely missed by his patients and his numerous friends.

J. MORGAN

Br. J. Derm. (1972), **87**, 288.

Society Proceedings

ROYAL SOCIETY OF MEDICINE

SECTION OF DERMATOLOGY

President—Dr H. R. VICKERS

Thursday, December 16th, 1971

Lymphocytoma Cutis.—Dr D. D. MUNRO and Dr A. du VIVIER.

A woman aged 61.

History.—Eighteen years ago she presented a nodular eruption on the face and the front of the neck. Since then the rash has been exacerbated in the summer and improved in the winter. Four years ago some nodules on the right temple remained throughout the year and gradually increased in size. There has been no irritation and the rest of the skin has not been affected. Throughout her life she has developed sunburn after relatively short exposure to sunshine. No family history of light sensitivity was recorded.

On examination.—Marked solar atrophy of the facial skin was present; some areas showed the yellowish, raised micropapules seen in severe atrophy. Superimposed on the atrophic change were many slightly translucent, pink nodules measuring up

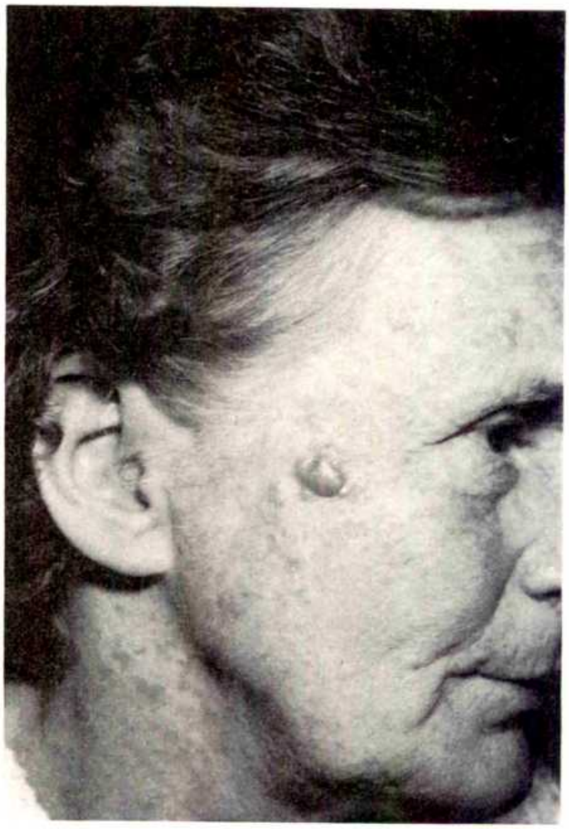

FIG. 1

to 1 cm in diameter. One group above the right eyebrow was especially prominent (Fig. 1). The patient was ginger haired, blue eyed and fair skinned: general examination revealed no abnormality.

Investigations.—Skin biopsies 18 years ago and recently showed a similar histology. The epidermis and papillary layer were normal but the mid and lower dermis showed a dense infiltrate of histiocytic reticulum cells interspersed with numerous small lymphocytes and a few eosinophils (Fig. 2).

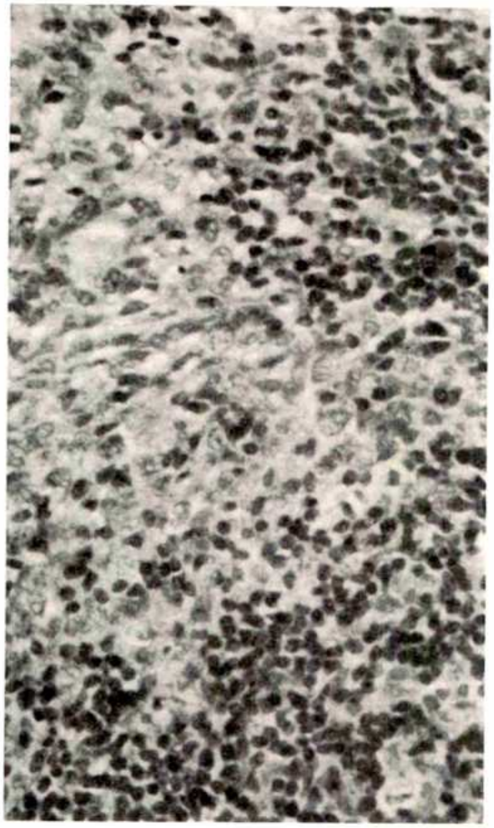
FIG. 2

A full blood count and E.S.R. were normal.

Comment.—Benign lymphocytoma cutis is frequently a light sensitive eruption which remits and relapses, though Beare (1968) thought this a feature more commonly seen in lymphocytic infiltration of the skin (Jessner). The long history in this very fair skinned patient with the development of more persistent nodules might be suggestive of a malignant change, but the histology continued to show a benign pattern with a " clear " zone in the upper dermis. The persistent nodules responded rapidly to irradiation (140 kV 2500 ad) but lymphocytic infiltrations are commonly radiation sensitive whether they are benign or malignant. The dual population of cells in the infiltrate (Hirsch and Lukes, 1965) and the sparse eosinophils (Höfer, 1956) are a feature of the benign localized form of the disease. The reduction in nodulation of the forehead which followed sunlight barrier preparations, including 5% para-aminobenzoic acid in alcohol and the application of corticosteroid creams also favoured a benign reaction.

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The following cases will be published in *Proc. R. Soc. Med.*

Chronic Repetitive Suppuration of the Skin Associated with a Serum Factor Affecting the Bactericidal Capacity of Polymorphonuclear Leukocytes.—Dr G. A. HODGSON and Dr EILEEN THOMPSON

Chloracne: Three Cases.—Dr N. E. JENSEN (for Dr I. B. SNEDDON and Dr A. E. WALKER).

Porphyria Cutanea Tarda, Carcinoma of Bronchus, Rheumatoid Arthritis, Right Hydronephrosis.—Dr C. R. NYMAN (for Dr M. FEIWEL).

Discoid Lupus Erythematosus, Fatty Infiltration of Liver (Alcoholic) and ?Porphyria.—Dr A. MACDONALD (for Dr M. FEIWEL).

Br. J. Derm. (1972) **87**, 290.

Books

Consultations in Dermatology with Walter B. Shelley (1972). Philadelphia: Saunders. Pp. 289, illus. Price £4.70.

It was a pleasure to review this book. It is a collection of 50 lecture-demonstrations, the patients being chosen because they illustrate common clinical problems or diseases of particular interest. 50 full page photographs show that good black and white pictures are better than colour reproductions in all but exceptional circumstances. 48 of these pictures cannot be faulted; only those of tinea versicolor and pityriasis rosea are below the very high standard of the others. The text is an intriguing distillate of clinical observation, pathology, immunology and therapeutics. Where treatment does not work the fact is clearly stated; where there is doubt this too is usually made clear, as in the section on the treatment of melanomata. Some dogmatism is inevitable in such a book but one wonders whether it is wise to treat tinea versicolor with indefinitely repeated whole-body applications of selenium sulphide shampoo, or to recommend surgery quite so strongly in the treatment of a patient with stasis dermatitis. Each demonstration concludes with a valuable selection of references, many from the 1970 or 1971 literature. In its turn each reference is concisely summarized in two or three lines.

Senior and junior dermatologists will enjoy this book and so will general practitioners. General physicians and surgeons could learn much from it; students should read selected parts of it. For nurses in general training many of the sections are too advanced but there is much to interest nurses if they are working on a skin ward. Buy this book.

R. E. BOWERS

Hazards of Medication. A Manual of Interactions, Incompatibilities, Contra-indications, and Adverse Effects. Edit. E. W. Martin and Ruth D. Martin (1971). Philadelphia: Lippincott. Pp. 895. Figures and tables. Price £13.75.

This is a large book but the reader should not allow himself to be put off by its size, the polysyllabics of the sub-title nor by a turgid style which reveals itself in the opening sentence of the preface. " This volume ", it says, " presents an overview of the numerous therapeutic, biopharmaceutic, legal and scientific requirements for safe and effective medication of the patient, a major function of the health care system ". It does just that and brings together in one valuable volume a vast array of data concerning drugs and their use. The senior editor's background as a Professor of Pharmacy at Columbia and as an executive of Lederle Laboratories ensures a panoramic view of drug science, spanning research and development, manufacture, distribution and storage on one hand and the clinical aspects of prescribing, laboratory control of dosage and variations in patient response on the other. Selected references follow each chapter.

A comprehensive table of contents and a good index, allied with the generous use of sub-headings in the text allow the reader to find specific information speedily and easily. Particularly valuable are 45 pages of tables printed in a distinct colour, detailing the impact of drugs on laboratory tests of blood and urine chemistry and over 400 pages of tables, again distinctly coloured, summarizing present knowledge of drug interactions.

The chapter on adverse drug reactions would be more valuable if its tables listing the causes of various " dermatomucosal " syndromes were less comprehensive. The clinician consulting the table on p. 350 listing photosensitizers, for instance, wants to know which 10 or 20 of the 150 substances listed are most commonly to blame.

However, this and other minor criticisms do not detract from the value of a book which can only be an asset on the shelves of any hospital library, pharmacy or clinical department.

HARVEY BAKER

The Third Conference on the Clinical Delineation of Birth Defects, Part XII. Skin, Hair and Nails. Edit. DANIEL BERGSMA (1971). London and Edinburgh: Churchill-Livingstone. Pp. 357; numerous illustrations. Price £10.00.

This well produced volume, sponsored by the National Foundation—March of Dimes, records the proceedings of the Conference held at the Johns Hopkins Hospital, Baltimore, in June 1970. Dr V. A. McKusick dedicates the volume to the memory of Jonathan Hutchinson and in doing so reminds us that the invaluable Hutchinson Collection was acquired by Johns Hopkins University in 1913, after both Royal Colleges in London had refused it.

Two-thirds of this volume is devoted to the papers presented at the Conference. Together they cover in somewhat random fashion a large variety of hereditary states in which the skin is in some way defective. The team of authors is international and most of their contributions are valuable; although some describe work which is now fairly well known to those interested in dermatological genetics, the majority present at least some new material, and a few are likely to revolutionize established concepts of familiar entities. Almost all the numerous black and white illustrations are of excellent quality. Those that portray the authors on each title page could perhaps have been omitted, pleasant though it is to see many old friends.

Of particular interest are the three different approaches to the investigation of congenital hair defects adopted by Algie Brown, Paul Porter, and Lowell Goldsmith and Howard Baden, and Reynold Gold's characterization of the abnormalities of keratin in hidrotic ectodermal dysplasia. The demonstration by John Voorhees that the XYY syndrome occurs more frequently in patients with nodulocystic acne than in controls raises fascinating speculations. Bill Reed writes well on the genetics of the photodermatoses.

The final third of the book consists of case reports, often quite brief, but well illustrated and documented.

This book is a gold mine for all who are concerned with the diagnosis and investigation of hereditary skin diseases.

ARTHUR ROOK

Correspondence

Zinc and Copper in Psoriasis

SIR,—Greaves (1972), who believes that serum zinc is related to the extent of skin involvement in psoriasis, has once more entered the lists on this subject and again has quoted his work (Greaves and Boyde, 1967) as if it were a valid statement of fact. He bolsters up his thesis by quoting his only protagonist on serum levels, namely Voorhees *et al.* (1969), whose 4 cases he now elevates to " an American study ". In our letter (Portnoy and Molokhia, 1971) we have been highly critical of his original paper (Greaves and Boyde, 1967) in which the Newcastle workers were adding " one drop " of heparin à la Mrs Beeton and this criticism has not been refuted. In a personal communication, Greaves (1971) has admitted that this was " unscientific ", his apologia being that this point was "completely overlooked " by him and that "that part of the paper was written by Boyde ". This frank admission cannot absolve Greaves from joint responsibility. Co-authors must mutually and equally accept the fame or blame for any scientific work.

With regard to our letter (Portnoy and Molokhia, 1972), the printers error in omitting the signs " more than " before 10% and " less than " before 5% in the tables, since corrected in an erratum (1972), may have given cause for Greaves to question its relevance, but we had already made it crystal clear in our earlier correspondence (Portnoy and Molokhia, 1971) as to our intent and indicated our results at that time. In fact the only reason for selecting the 2 groups of patients in this fashion was to satisfy Greaves' own criteria for severe and mild psoriasis respectively.

Greaves now asks why we object to the use of his 1967 results as control for work done 4 years later and wants us to provide him with any evidence that the control population in Newcastle has changed in that period. We have no such evidence but would refer him to the work of Withers *et al.* (1968), for which Greaves incidentally gave the wrong reference. These workers, whose results agree with ours, repeated their control zinc measurements in a London population within a year and registered a rise of nearly 15%. As Greaves is comparing various groups of the Newcastle population at a 4 years interval, it is not up to us to show that there have been any environmental changes but up to him to show that there have not. However, this is not the main reason why we reject such a comparison although it is a valid one. It is simply because, again on his own admission, Greaves (1971) has modified his method and now uses a measured amount of heparin or sodium citrate. In view of these modifications, it is now mandatory for him to repeat his earlier studies on psoriasis using contemporaneous controls and a technique which satisfies his own demand for " further meticulous work . . . and experimental conditions ". Only this can bring the results of our two laboratories closer.

Skin Hospital
Manchester

Benjamin Portnoy
Mohamed Molokhia

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The Effect of a Medicated Wash on Acne

Sir,—I have made a clinical assessment of the efficacy of the Medicated Wash referred to by Dr Cunliffe and others (1972).

The object of the trial was to see whether the use of this cleansing and antiseptic preparation enhanced the effect of other local treatment, in this case with Clearasil Cream Medication. A comparison was made with plain, unmedicated soap (Simple Soap) and with a medicated soap.

Throughout the trial patients applied the medicated cream twice daily after cleansing the skin with one or other preparation. Each cleanser was used in this way for a month at a time and then changed for the next one. The order in which they were taken into use was randomized.

Results were assessed at the end of each monthly period and were recorded as " improved ", " unchanged " or " worse ". Since acne may vary from day to day the patient was asked to assess his progress over the month in addition to the assessment made by the doctor at the time of examination. Doctor's and patient's own assessment were given equal value so that each patient rated two scores.

Fifty patients entered the trial and forty-one completed it. The results showed that under these conditions the use of the medicated wash was followed by significantly greater ($P < 0\cdot05$) improvement than the unmedicated soap.

	Medicated Wash	Soap
Improved	60 (73·2%)	49 (59·7%)
Unchanged	13 (15·9%)	24 (29·3%)
Worse	9 (11%)	9 (11%)

During the trial one patient found the wash dried the skin excessively and one observed slight stinging when it was applied after shaving.

The Middlesex Hospital
London, W.1

F. Ray Bettley

REFERENCE

Cunliffe, W. J., Cotterill, J. A. & Williamson, B. (1972) The Effect of a Medicated Wash on Acne, Sebum Excretion Rate and Skin Surface Lipid Composition. *Br. J. Derm.*, **86**, 311.

Quarterly Review

DRUG REACTIONS XIX

Adverse Cutaneous Reactions to the Penicillins—Ampicillin Rashes

The penicillins are the most widely used antibiotics, and there are few individuals who have not been exposed to them. The members of this group of drugs are derived from 6-aminopenicillanic acid and include benzylpenicillin, methicillin, cloxacillin, flucloxacillin, ampicillin and carbenicillin.

The penicillins possess a very low direct toxicity, the most frequent being due to hypersensitivity. It is now generally accepted that the principal antigenic determinant of penicillin is not benzylpenicillin itself, but rather its degradation products. It has been suggested that in allergic individuals the main offenders which function as haptens are benzylpenicillenic acid and benzylpenicilloic acid, the principal antigen being penicilloyl-protein. (Levine, 1964). Since the synthetic penicillins are formed by combinations of various side chains to the 6-aminopenicillanic acid nucleus, cross hypersensitivity occurs between all penicillins.

The incidence of hypersensitivity reactions increases with prolonged therapy, or high total dose. Estimates of overall incidence of reactions to penicillin varies from 1 to 10%, and the incidence of fatality in severe reactions is estimated to be between 9 and 13% (Shelley, 1963; Hewitt, 1963). In the United States of America it has been reported that between 100 and 300 deaths each year can be attributed to penicillin (Feinberg, 1961). The parenteral route of penicillin administration more frequently leads to allergic reactions than does oral treatment. Opinions vary, but there is a strong feeling that atopic individuals are more susceptible to anaphylactic reactions (Jillson and Porter, 1965; Rosenthal, 1958).

Hypersensitivity reactions are usually the result of medical use of commercial preparations of these antibiotics. Contact with penicillin can occur without the patient ever having been treated with it. Penicillin is added to animal feeds to promote growth, and is also given to cows for mastitis, consequently, milk and other dairy products are often contaminated with the antibiotic, and provide a source of exposure to it (Jillson and Porter, 1965). In hospitals contamination of syringes may easily occur, since it is practically impossible to destroy the antigenic principles of penicillin with boiling, or steam sterilization.

The cutaneous manifestations of penicillin hypersensitivity are non-specific. Urticarial, erythematous or morbilliform eruptions are commonest. Urticaria may occur as part of an immediate anaphylactic reaction, or on its own several days after the commencement of penicillin therapy. It may also form part of a serum-sickness syndrome which is manifest by fever, pruritus, oedema, painful swollen joints, lymphadenopathy and eosinophilia. Occasionally a bullous eruption may appear around the wrists and ankles, sometimes unilaterally, while the other areas of the body present only an urticarial eruption (Bolgar *et al.*, 1960). Repeated exposure to penicillin, as in milk products, may cause chronic urticaria. Erythema, when present, may be scarlatiniform, and non-thrombocytopenic purpura has been observed (Hewitt, 1963). It has been stated that allergic reactions to penicillin appear more frequently in patients with an active dermatophyte infection and trichophyton sensitivity (Blum and de Weck, 1966). Pruritus ani occurs in some patients and is thought to be due to antibiotic-induced alteration of the bowel flora (Feinblatt and Feinblatt, 1960). Other less common cutaneous side effects include erythema nodosum (Levine, 1972), exfoliative dermatitis (Hewitt, 1963), fixed drug eruptions, which are occasionally bullous (Derbes, 1964), erythema multiforme (Rees, 1964; Levine, 1964), a systemic lupus erythematosus-like syndrome (Gold, 1951), polyarteritis nodosa (Feinberg and Feinberg, 1956) and toxic epidermal necrolysis (Beare, 1962).

It is worthy of mention that the cephalosporins, which are derivatives of 7-aminocephalosporanic acid, are now recognized to exhibit cross hypersensitivity with the penicillins (Thoburn *et al.*, 1966). The cephalosporins must thus be used with caution in the treatment of patients with a history of allergic reactions to penicillins.

Ampicillin Rashes

Ampicillin was introduced into clinical medicine in 1961, and is by far the most widely used of the synthetic penicillins. The major advantage it has over benzyl penicillin and phenoxymethyl penicillin being that it is active against gram negative bacteria, whilst still being bactericidal.

Two main groups of rashes occur in the course of ampicillin therapy. Firstly, a hypersensitivity rash which is urticarial and similar to the rash associated with a true penicillin hypersensitivity. This may appear soon after the onset of treatment, or may be delayed for as long as 3 weeks. The incidence of urticaria from ampicillin is no commoner than with other penicillins (Knudsen, 1969). It may be associated with angioneurotic oedema or as part of a more widespread serum-sickness syndrome. Anaphylactic reactions to ampicillin have only rarely been reported. Secondly, and much more commonly, an erythematous, dull red, macular or maculopapular rash, less irritant than urticaria develops. It starts most commonly on the extensor aspects of the limbs with accentuation over elbows and knees, and spreads symmetrically to most parts of the body becoming confluent. The rash may appear as early as the first day of treatment but usually between 5–14 days, thus it is often first noted after ampicillin therapy has been stopped. Mild rashes clear within a few days, but more severe rashes, which are often purpuric, may last a week or more before desquamating and leaving considerable residual staining. Involvement of the mucous membranes, particularly, the palate, may occur and occasionally slight fever is present.

A high incidence of this erythematous type of rash was noted in infections mononucleosis patients treated with ampicillin (Patel, 1967). He noted an incidence of 100%, Pullen *et al.* (1967) noted 95%, whilst Brown and Kanwar (1967) recorded an incidence of 69%.

Klemola (1970) showed an increased incidence of ampicillin rashes in patients with cytomegalo virus mononucleosis treated with this drug. All 5 patients (100%) treated, developed an ampicillin rash, whereas only 2 out of 23 patients not exposed to ampicillin had skin manifestations. This condition is similar to infectious mononucleosis but with a negative heterophil agglutination test and without tonsillitis or lymphadenitis.

More recently, Cameron and Richmond (1972) noted 10 out of 12 of their patients with lymphatic leukaemia developed a typical ampicillin rash when given ampicillin, compared with a control group of 85 patients with other diseases in which only 2 patients developed a rash.

An increased incidence of ampicillin rashes in patients who are also receiving allopurinol has been shown (Shapiro *et al.*, 1972). They recorded an incidence of 22·4% in patients receiving the two drugs compared with 7·5% in those patients only receiving ampicillin. They were unable to state whether the potentiation could be ascribed to hyperuricaemia as data of uric acid levels were not available. However Fessel (1972) has studied 124 patients with asymptomatic hyperuricaemia and compared them with 224 matched normouricaemic patients. He found that 14·1% compared with 4·9% gave a history of allergic reactions to penicillin. Although he did not specifically state the figures for ampicillin, he suggested that the basis of the increased number of ampicillin rashes was not treatment with allopurinol but rather an altered immunological reactivity in hyperuricaemia.

Lee Potter (1972) has recorded an ampicillin eruption in a patient with stage 4 reticulosarcoma. It has also been noted that patients with viral infections appear to develop a typical ampicillin rash more readily than those being treated for a bacterial infection.

The cause of this type of rash is unknown. It is uncertain if the eruption is an allergic or toxic manifestation, but several theories have been suggested. The traditional view has been that the penicilloyl group is the major allergic component in penicillin allergy. As it is common to all the semi-synthetic penicillins, it was reasonably assumed that ampicillin rashes would, like penicillin, be due to hypersensitivity to this component. Pullen *et al.* (1968) pointed out that many ampicillin rashes may not be due to true 6-aminopenicillanic acid nucleus hypersensitivity because benzyl and phenoxymethyl penicillins did not produce the same high incidence of rash in infectious mononucleosis yet this nucleus was common to all three drugs.

Knudsen (1969) and Pullen (1969) concluded that most erythematous rashes reported during ampicillin therapy were apparently ampicillin-specific and that patients reacting to the drug in this way should not necessarily be assumed to be sensitive to penicillin. Clinical support for this came from Nazareth (1971). He gave graduated doses of ampicillin to patients some months after their ampicillin reactions, but still during their attack of infectious mononucleosis. No rash or other hypersensitivity developed in all 10 patients tested.

It is possible that impurities produced in the manufacturing processes of ampicillin play a part in the development of this rash. Knudsen *et al.* (1970) showed that purification of ampicillin with respect to protein impurities, significantly reduced the incidence of rashes in treated patients. In Holland, Hendriksen (1972) showed a higher incidence of rash when ordinary ampicillin, rather than a similar product free from protein particles, was administered.

Lymphocytes have a vital role in antibody formation and cell mediated immunity (Ford, 1967). It was suggested that patients with infectious mononucleosis, (which is characterized by production of abnormal antibodies and abnormal lymphocytes), might be hypersensitive to ampicillin because of abnormal lymphocyte function (Pullen, 1971). Cameron and Richmond (1972) suggested that the striking incidence of rash in lymphatic leukaemia patients treated with ampicillin may also be due to the alteration of normal immune mechanisms resulting from abnormal but immunologically competent lymphocytes.

There are many clinical findings which seem to mitigate against hypersensitivity as a main cause. The relatively short incubation period, the infrequency of anaphylaxis, the inability to demonstrate reaginic antibodies (Knudsen, 1969), negative lymphocyte transformation tests (Sarkany, 1968) and the fact that patients do not necessarily develop further rash if they are at a later date given ampicillin. Rash has cleared even though the patient has continued taking ampicillin (Patel, 1967; Crow, 1970), although, this may have been because of development of a temporary latency with regard to hypersensitivity, and need not mean that ampicillin may be given subsequently with impunity (Pullen, 1971).

Whatever the cause, whether it be allergic or toxic, certain important clinical points can be made. The use of ampicillin in infectious mononucleosis and cytomegalovirus mononucleosis, should be avoided, and its use in lymphatic leukaemia and reticulosarcoma should be restricted. Extra care should be used if prescribing it to patients with hyperuricaemia, or if patients are receiving allopurinol. An erythematous reaction to ampicillin does not necessarily mean that the patient is allergic to other penicillins.

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News and Notices

WILLAN HOUSE APPEAL FUND

" The founder of modern dermatology ", Robert Willan, was born at " The Hill ", Marthwaite, near Sedbergh, Yorkshire and died 160 years ago. " The Hill ", an old farmhouse dating from the 17th century is a " listed building " and is owned by Mr L. P. Madge, senior Biology master, Sedbergh School. He and his wife have carried out a great deal of restoration to the house and garden, but the adjacent barn, which has a dated Willan plaque on a gable-end, and which is said to be of outstanding architectural interest, has collapsed. A local builder has estimated that its repair would cost £1,500.

Mr Madge states " My wife and I would certainly be very willing that a suitable plaque should be placed on the house and are most anxious that members of the Association and other duly qualified dermatologists shall have free access to the house, provided, in so far as is possible, we were warned of pending visits. Access *in perpetuo* raises all sorts of legal difficulties, I am sure . . . all that we can say is that we would do our very best to ensure that if we sold the property the future owner would be asked to continue the above access arrangements."

Those wishing to help this worthy appeal should send their contributions to our Treasurer, Dr O. L. S. Scott, South Lodge, Wimbledon Common, London, S.W.19.

Postgraduate Medical School, University of Cambridge
Progress in the Biological Sciences in Relation to Dermatology

The fourth Course will be held in Cambridge from September 24th to 29th, 1973. This Course, like its predecessors, is designed to present at a fairly advanced level an account of progress in selected fields of biological science relevant to dermatological practice and research. Each session will be devoted to different approaches to a disease or a group of disease. Enquiries should be addressed to J. W. Woodcock, M.A., Secretary, Cambridge University Medical School, Hills Road, Cambridge.

Investigative Group of The British Association of Dermatology

The next meeting of the Group will be held at the Edward Lewis Theatre, Middlesex Hospital School on Saturday February 3rd, 1973.

Those members wishing to submit papers should send abstracts in the form prescribed to: Dr C. N. D. Cruickshank, M.R.C. Skin Unit, The Medical School, University of Birmingham, Birmingham 15, not later than October 14th, 1972.

International Mycology Symposium

Under the joint sponsorship of the International Society of Tropical Dermatology and the Roumanian Dermatological Society, a Symposium on Medical Mycology will be held on September 20–22nd, 1973 in Bucharest. The following are the main themes:

1. Recent advances in the epidemiology of mycoses.
2. New investigative methods related to the etiopathogenicity and taxonomy of mycoses.
3. New trends in the clinical manifestation of mycoses.
4. Therapeutic results with new drugs in the treatment of superficial and deep mycoses.
5. Round Table Conference: Training in medical mycology (graduate and post-graduate).

Those interested in participating should write to: Dr I. Gojocaru, Uniunea Societatilor de Stiinte Medicale, 8 Str. Progresul, Bucharest, Roumania.

European Society for Paediatric Dermatology

The need for a Society to discuss and advise on Paediatric Dermatology in Europe emerged from informal meetings which were held during the recent International Dermatological Congress at Venice, and it is hoped to hold the inaugural meeting of the Society in the Summer of 1973.

The membership of the Society will be open to all who are interested in diseases of the skin in infancy and childhood, who are resident in Europe or the countries of the Mediterranean Ltitoral. Membership will not be restricted to dermatological paediatricians. Geneticists, pathologists and those engaged in fundamental developmental research in biology will be welcomed.

Would those interested get in touch with Dr E. J. Moynahan, Hospital for Sick Children, Great Ormond Street, London, W.C.1.

European Society for Dermatological Research

The third meeting of the European Society for Dermatological Research will take place in the RAI-Congrescentrum in Amsterdam on April 25–26th, 1973.

Attendance at the scientific sessions will be open to all members, contributors, introduced by members, and guests. Non-members who wish to present a paper are kindly requested to send an abstract to the Secretary. They will be invited to attend the meeting either as contributors or guests.

Abstract forms are available on simple request to the Secretary.

All abstracts should be sent to Michel Prunieras, Secretary, Fondation Ad. de Rothschild, 29 rue Manin, 75019 Paris, before December 15th, 1972, Deadline.

Appointments in the E.E.C.

Groningen

Dr A. H. Klokke has succeeded Dr M. Ruiter as Professor of Dermatology, University of Groningen.

CORRIGENDUM

The mouse represented in the left hand half of Figure 6, page 477, should have appeared as a third mouse in Figure 5.

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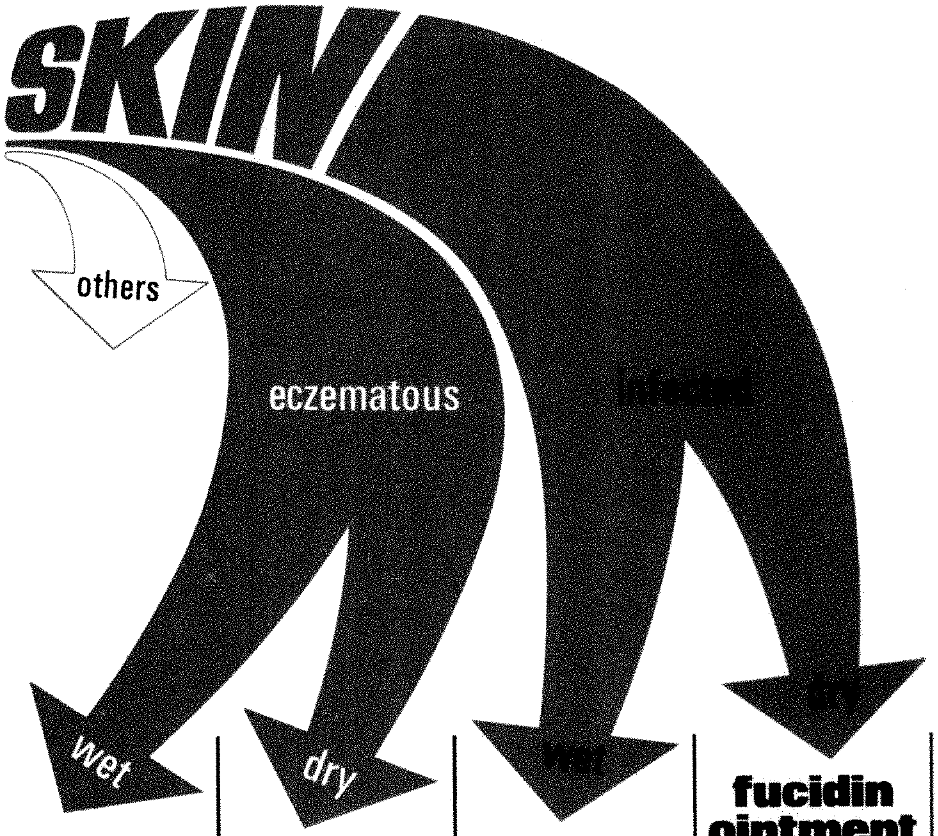

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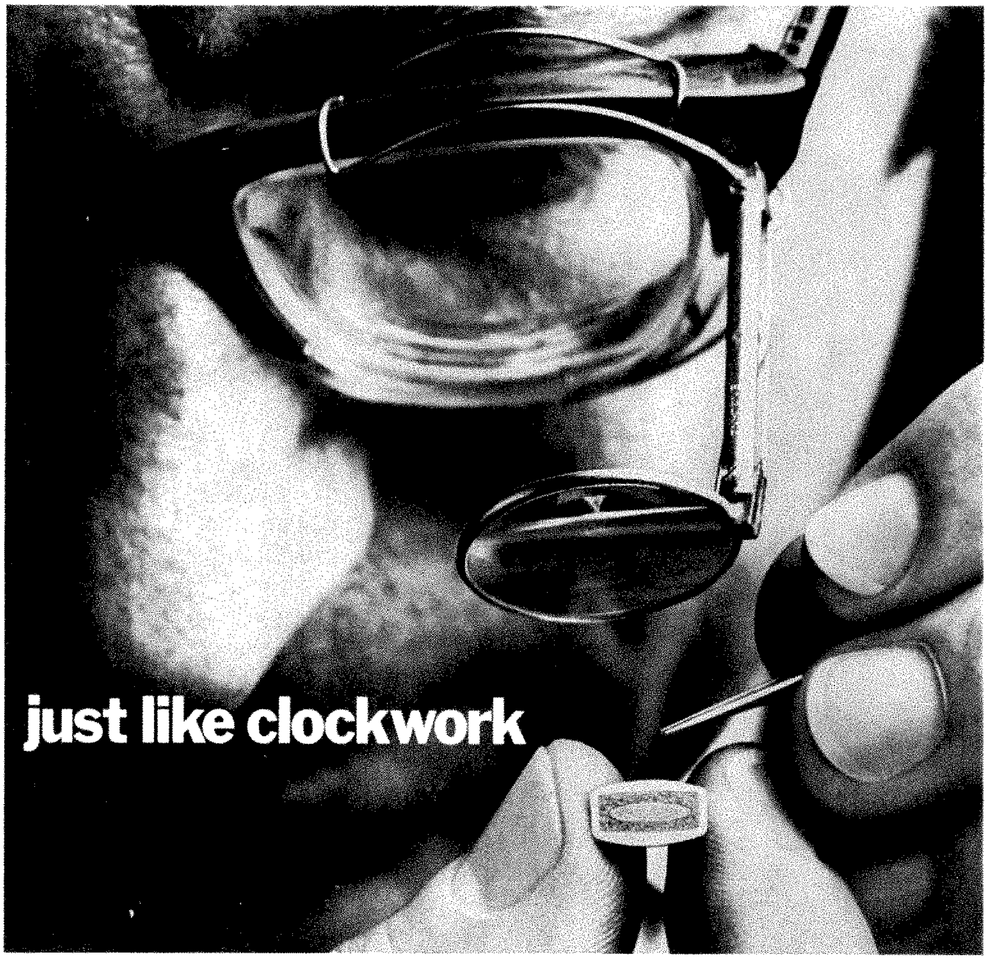

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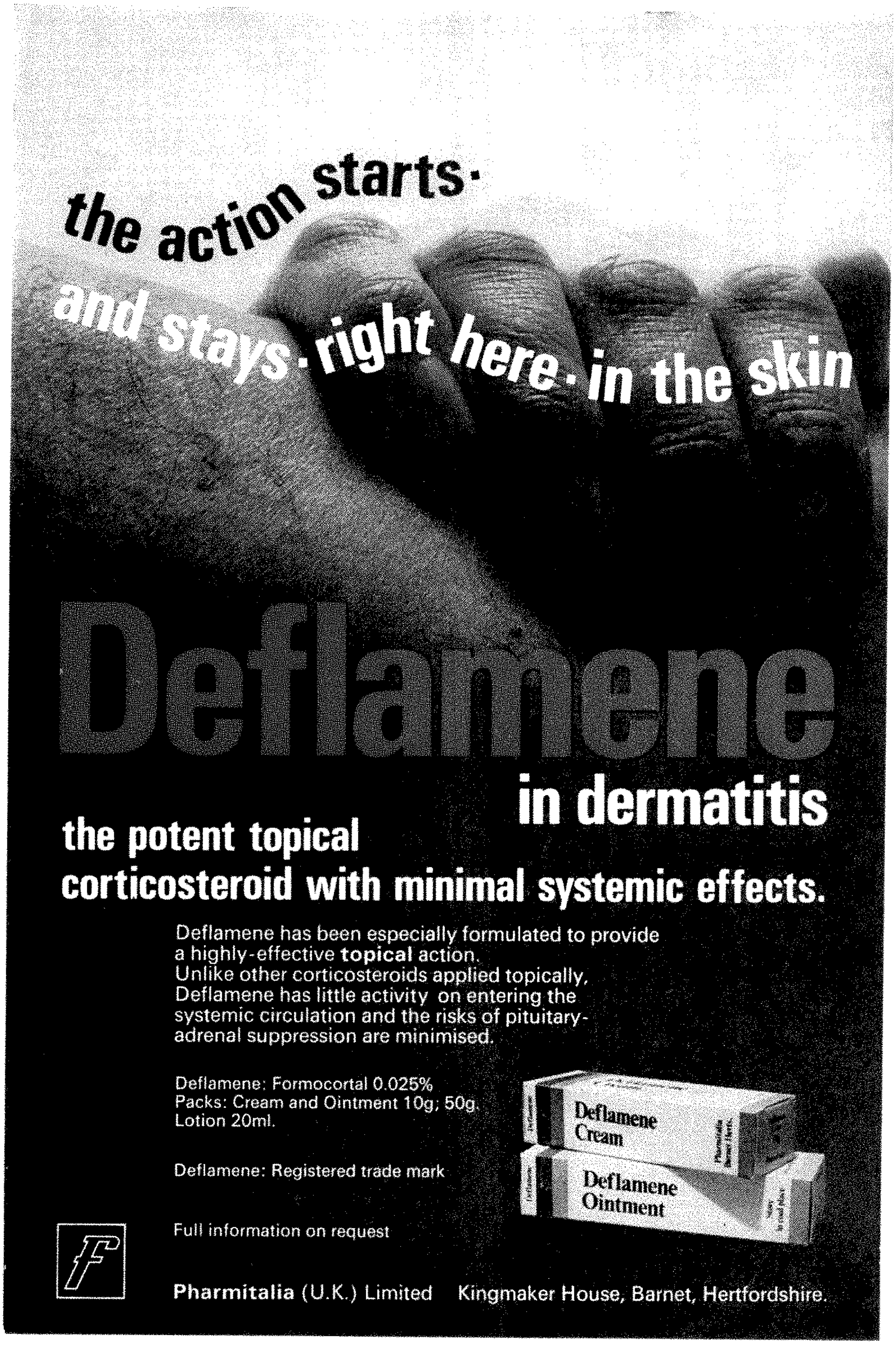

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No.8.	Microbial Skin Disease

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CONTENTS (*continued*)

Comment

The Nature, Pathogenesis and Staining Properties of Amyloid
MARTIN M. BLACK 280

Obituaries

JOHN LEWIS FRANKLIN 284
E. C. RITTER 285
GILBERT HENLY 286
G. H. RITTERMAN 287

Society Proceedings

Royal Society of Medicine, December 16th, 1971 288

Books

Consultations in Dermatology with Walter B. Shelley; Hazards of Medication (Martin and Martin (Edit.)); The Third Conference on the Clinical Delineation of Birth Defects, PartXII. Skin, Hair and Nails (Bergsma (Edit.)) 290

Correspondence

Zinc and Copper in Psoriasis
BENJAMIN PORTNOY and MOHAMED MOLOKHIA 291
The Effect of a Medicated Wash on Acne
F. RAY BETTLEY 292

Quarterly Review

Drug Reactions XIX. Adverse Cutaneous Reactions to the Penicillins —Ampicillin Rashes.
JOHN ALMEYDA and A. LEVANTINE 293

News and Notices 297

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

18 DEC 1971

PAGE

Clinical and Laboratory Investigations

The Control of Epidermal Thickness
William S. Bullough 187

Electronmicroscopic Study of Hypopigmented Lesions in Leprosy. A Preliminary Report
C. K. Job, Ammu Nayar and J. S. Narayanan 200

The Role of the Circulation in Measurements of Skin Conductivity
Ann Woolley-Hart 213

Changes in the Fatty Acid Composition of the Phospholipids, Triglycerides and Free Fatty Acids with Depth in the Cow Snout Epidermis
V. J. W. Long 227

Allergic Contact Dermatitis from Sesquiterpenoids of Plants. Additional Allergenic Sesquiterpene Lactones and Immunological Specificity of Compositae, Liverworts and Lichens
J. C. Mitchell, G. Dupuis and T. A. Geissman 235

Seborrhoeic Dermatitis of the Beard
John A. Parrish and Kenneth A. Arndt 241

Dermatitis in Man and in a Dog Caused by the Mite *Cheyletiella yasguri* Smiley
E. J. M. Bakkers and A. Fain 245

Pharmacology and Treatment

Deficient Folate Activity during Treatment of Psoriasis with Methotrexate Diagnosed by Determination of Serine Synthesis in Lymphocytes
J. Ellegaard, V. Esmann and L. Henriksen 248

Effect of Retinoic Acid in Psoriasis, II. Long Term Study
Angus Macdonald, R. M. H. McMinn and Lionel Fry . . . 256

Studies on Low Dose Chloroquine Therapy and the Action of Chloroquine in Symptomatic Porphyria
J. J. F. Taljaard, B. C. Shanley, E. G. Stewart-Wynne, W. M. Deppe and S. M. Joubert 261

Intermittent Chemotherapy in the Treatment of Tuberculosis Cutis. A Preliminary Report
V. S. Rajan and Y. S. Goh 270

Case Report

Localized Acneiform Eruption Following Cobalt Irradiation
Kenneth M. Stein, James J. Leyden and Herbert Goldschmidt 274

(*Continued on* p. iii *of cover*)

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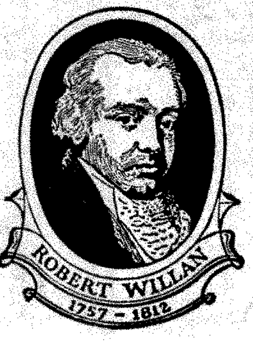

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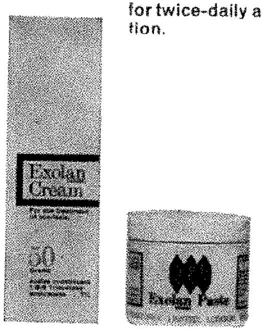

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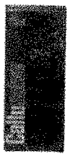

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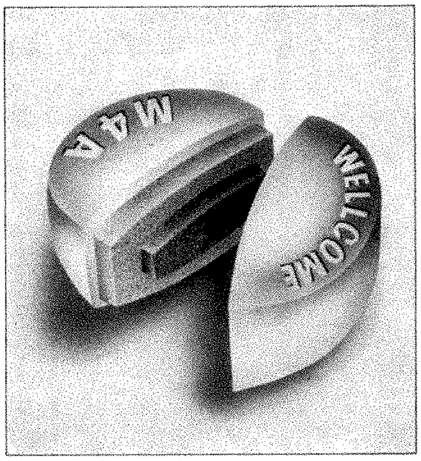

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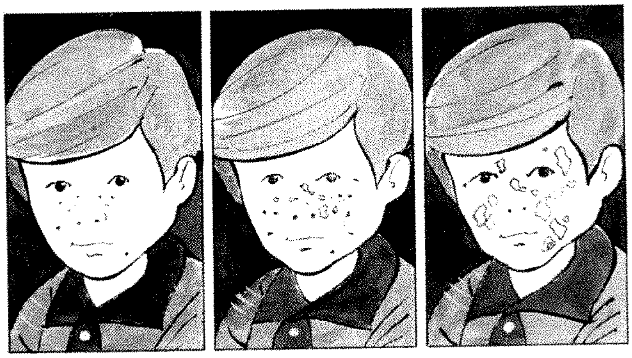

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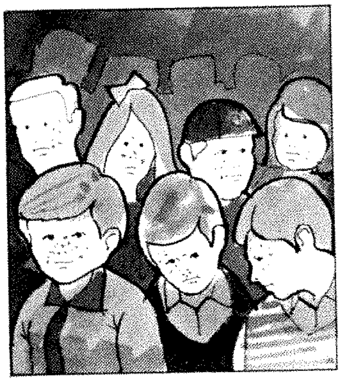

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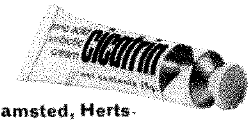

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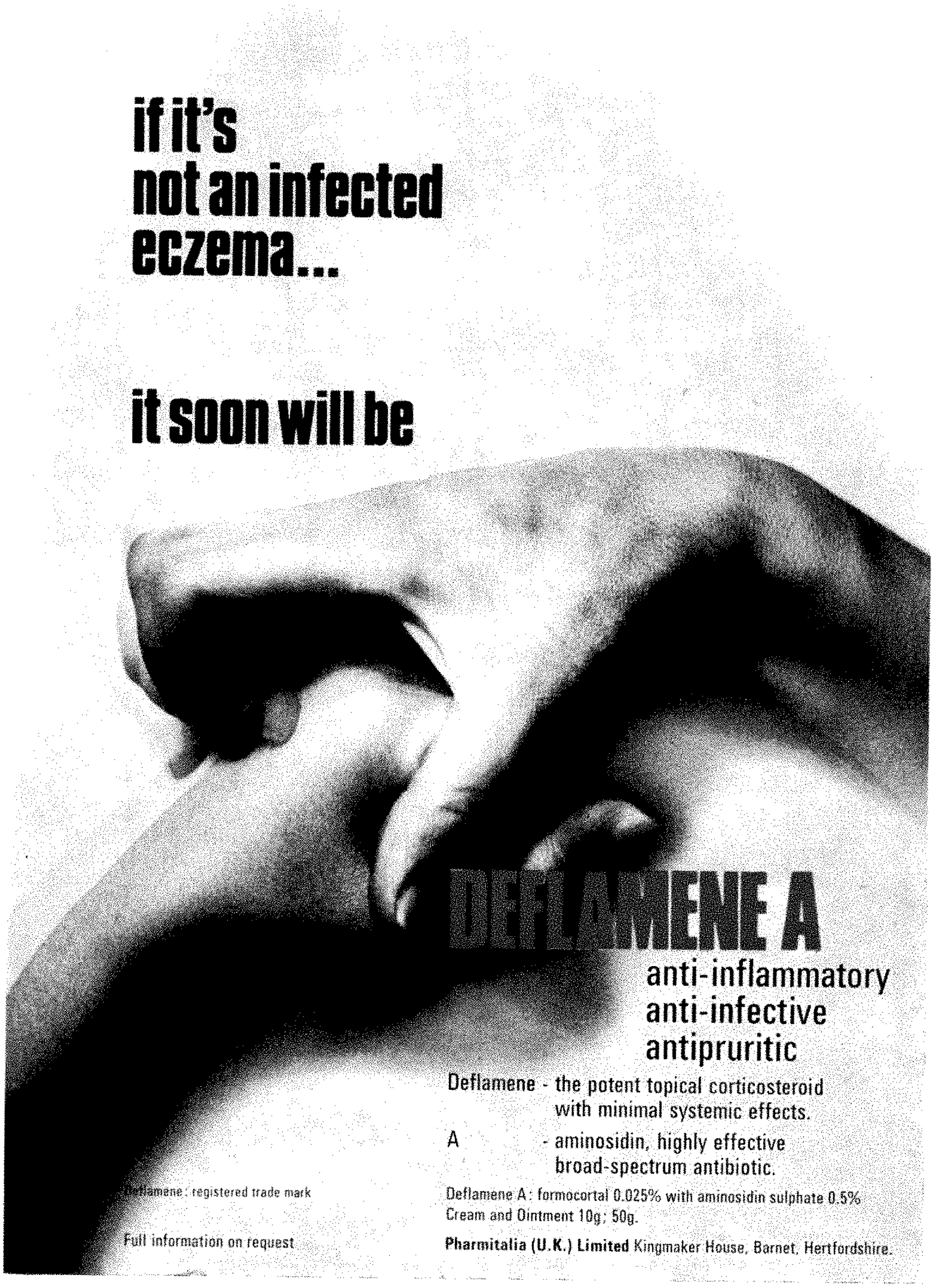

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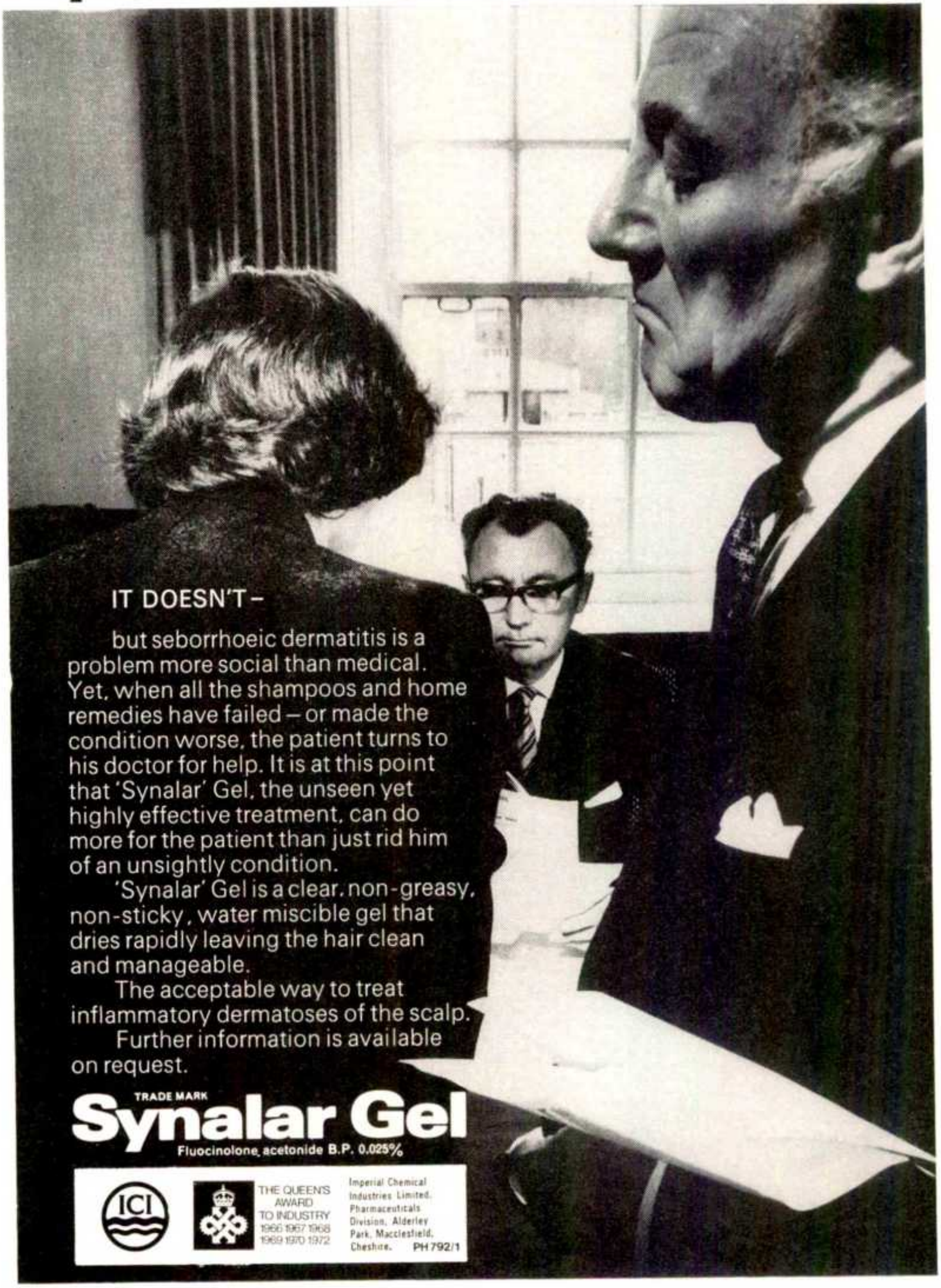

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University of California, U.S.A., and Institute of Dermatology,
St John's Hospital, London

HEREDITARY INFLAMMATORY VASCULITIS WITH PERSISTENT NODULES

A GENETICALLY-DETERMINED NEW ENTITY PROBABLY RELATED TO LUPUS ERYTHEMATOSUS

WILLIAM B. REED,* ROBERT F. BERGERON,† DENNY TUFFANELLI,‡ AND
EDWARD WILSON JONES,§

Summary.—A family of 3 generations is reported, in which several members had had recurrent nodules over the limbs since early childhood. Special features were: aggravation by exposure to sunlight, and complete suppression of the lesions with chloroquine therapy. Histological studies of the nodules showed a lymphocytic vasculitis without necrosis, extending deeply into the fat. The possibility that this is a particular familial form of lupus erythematosus profundus is discussed.

We would like to report a family of 3 generations (Fig. 1) in which recurring nodules had been present in the skin and the subcutaneous tissue since birth or shortly afterwards. These lesions were of 2 types: (1) Multiple, small to medium-

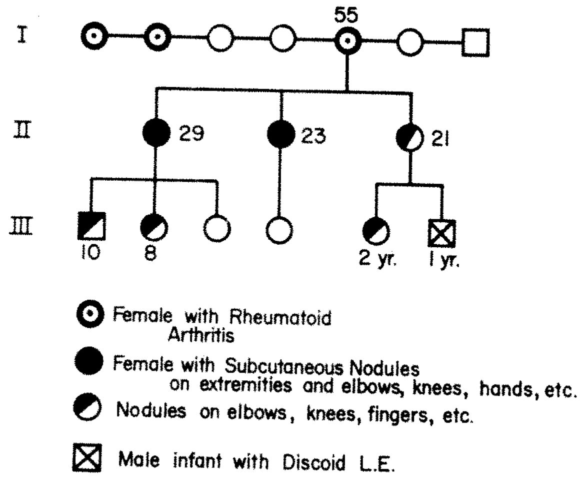
I
55
II
29
23
21
III
10
8
2 yr.
1 yr.
Female with Rheumatoid Arthritis
Female with Subcutaneous Nodules on extremities and elbows, knees, hands, etc.
Nodules on elbows, knees, fingers, etc.
Male infant with Discoid L.E.

FIG. 1.

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§ Department of Histopathology, St. John's Hospital, London.

sized nodules on the arms, legs and buttocks, similar in aspect to those of lupus erythematosus, (L.E.), except that these nodules were more profuse and persistent, and did not ulcerate; (2) Multiple, larger, rather firm, erythematous nodules (resembling rheumatoid nodules) occurring over the bony prominences.

Recently, detailed accounts of the relatively uncommon condition, L.E. profundus, have been published by Fountain (1968) and Tuffanelli (1971). In addition, Winkelmann (1970) has described an ulcerosclerotic variant of L.E. profundus which characteristically affects the gluteal region.

The patients we report may be examples of another, genetically-determined variant of L.E. profundus, but the exact status of these patients is as yet uncertain.

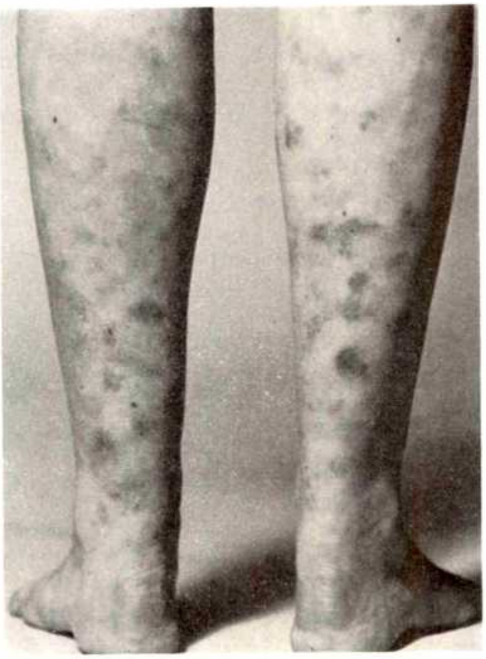

FIG. 2.—Multiple deep nodules of the legs (the proband II–1).

CASE HISTORIES

We have personally confirmed the 3 generation involvement in this family (Fig. 1).

In the first generation there were 6 women and one man. The grandmother (I–5), aged 55, has had typical rheumatoid arthritis since 1954, involving the hands, right foot and right shoulder. She has had nodules over the knees and elbows for as long as she can remember. At present she is receiving 10 mg of prednisone daily. Her mother and father had no signs of rheumatism and no subcutaneous nodules.

Her 2 sisters (I–1 and I–2) were not seen personally, but both have rheumatism. One, aged 60, has arthritis of the spine of unknown duration, while the other has typical rheumatoid arthritis, especially involving the hands. Neither of the sisters is known to have subcutaneous nodules, rheumatoid or otherwise.

Her daughter (II–1), aged 29, is the proband, Fig. 2, 3, and 4. She gave a history of recurrent nodules over the hands, elbows and knees all her life. In the past 3 years the lesions had become more widespread, and numerous scattered lesions had developed over the arms and legs on both the flexor and extensor surfaces. They may have been aggravated by sunlight, since 2 weeks of sun in Los Angeles were followed by a flare of the lesions on the hands and legs. The nodules were resistant to systemic cortico-steroid therapy and topical medicaments. She has had no arthritis, and she maintained that she was otherwise in good health. The nodules were of a reddish to purplish hue and were deeply set in the skin and subcutaneous tissue; no lichenoid features, follicular plugging or scaling were apparent. On one occasion she had a weakly reactive L.E. clot test and rheumatoid factor (Table I). The serum proteins, the W.B.C., the E.S.R., P.B.I., fibrinogen test, partial thromboplastin test and antinuclear antibody test were normal in May 1970.

A deep punch biopsy from a nodule of the thigh was taken to include fat. There was no difficulty in healing, in contrast to that sometimes experienced in L.E. profundus. A pronounced perivascular lymphocytic infiltrate with oedema was present throughout the

TABLE I. *Immunological Studies of Family*

Family	Rheumatoid Factor	Antinuclear Factor	Anti-DNA	Anti-RNA	L.E. Factor
I–5	Positive	Positive	Negative	Negative	Negative
II–1	Positive to negative	Positive	Negative	Negative	Sl. positive to negative
II–2	Negative	Negative	Negative	Negative	Negative
II–3	Negative	Positive	Negative	Negative	Negative
III–1	Negative	Negative	Negative	Negative	Negative
III–2	Negative	Negative	Negative	Negative	Negative
III–3	Negative	Negative	Negative	Negative	Negative
III–4	Negative	Positive	Negative	Negative	Negative
III–5	Negative	Negative	Negative	Negative	Negative
III–6	Negative	Negative	Negative	Negative	Negative

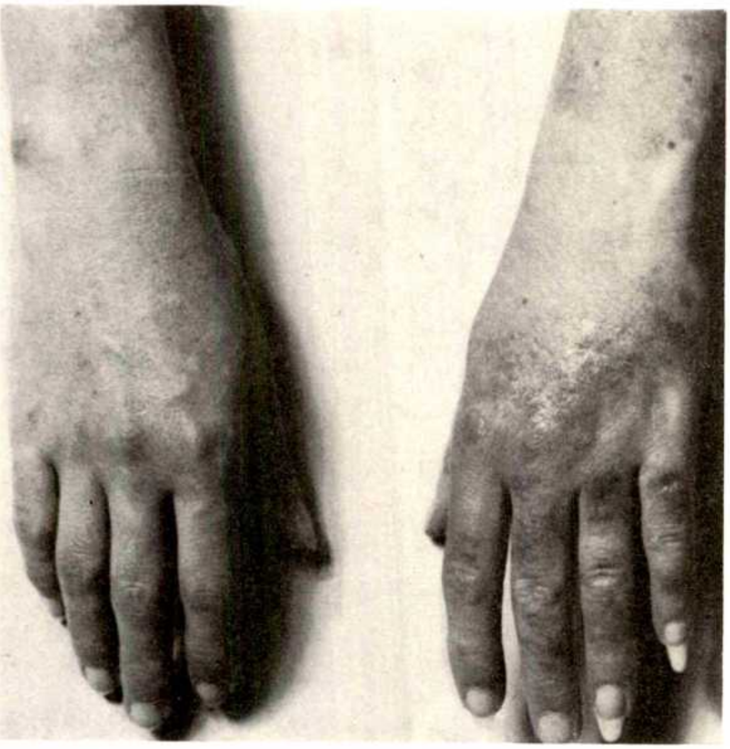

FIG. 3.—Nodules over the backs of hands and fingers. The left hand is much more severely affected than the right (the proband II–1).

skin. The infiltrate was especially intense in the lower dermis and in the vicinity of the sweat coils, and it extended into the fat. The affected capillaries tended to be dilated, and some showed endothelial swelling. However, the larger vessels in areas of infiltrate were intact, and their walls were not invaded by inflammatory cells. No fibrinoid necrosis of capillaries or leucocytoclasis was observed. A small proportion of larger mononuclear and histiocytic cells was also present in the infiltrate, but mast cells were sparse. Although well-marked foci of lymphocytic infiltrate were present between the fat cells, no areas of foreign body granuloma or fat necrosis were found (Fig. 4). The presence of mucinous oedema separating the intact collagen bundles was confirmed by

Alcian blue stain. No significant changes were detected in the basement membranes of the epidermis or vessels with the PAS stain.

The patient was given chloroquine (250 mg daily) for one-and-a-half-months and had complete clearance of all her lesions of arms and legs. Three subsequent short episodes rapidly cleared with chloroquine for 3 or 4 days only. The nodules on her knees, elbows and hands have also cleared with chloroquine. The dramatic disappearance of the nodules without any residual scarring after only a few weeks of chloroquine was similar to that observed in the patients described by Tuffanelli (1971), although these received the antimalarials for a longer time.

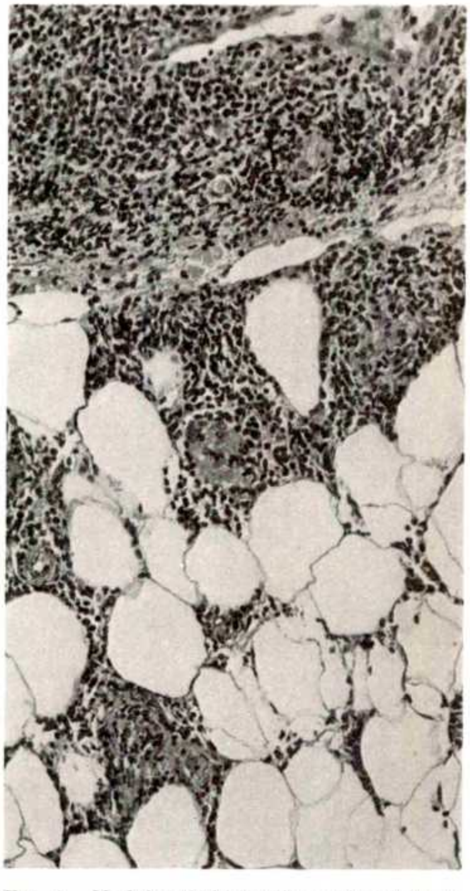

FIG. 4.—Nodule of thigh (the proband II–1). A heavy infiltrate of lymphocytes is present between the fat cells.

Her son, aged 10 (III–1), and daughter, aged 8 (III–2), are in good health except that they also have nodules on some joints. A nodule on the son's elbow bled when injured (Fig. 5). According to the mother (II–1) these nodules waxed and waned, especially in the summer. The children have no signs of rheumatism, or known sunlight sensitivity.

A biopsy from the son's elbow (III–1) showed a well-marked lymphocytic infiltrate surrounding the small dermal vessels and the skin appendages (Fig. 6). The vessels in the subpapillary zone tended to be dilated (Fig. 7), and in places there was slight swelling of the endothelial cells. The epidermis and the basement membrane appeared unaffected. The infiltrate was composed mainly of small lymphocytes with a few large lymphocytes and histiocytes. No fibrinoid deposits in or around the vessels were detected with the PTAH and PAS stains. The dermal elastica pattern was unchanged. No significant changes were seen in the small amount of fat included in the section.

A small biopsy of a nodule from the daughter (III–2) showed similar inflammatory charges in the upper dermis.

Another sister, aged 25 (II–2), also had nodules over the joints from early life; but when she was 7 or 8, she developed deep-seated purplish nodules over the calves, which persisted until about the age of 12. The arms were unaffected. There have since been intermittent flares, with intervals of freedom. Sun exposure was thought to have precipitated some of the attacks. In the summer of 1970 a few fresh nodules developed on the arms as well as the legs, but these cleared rapidly with a short course of chloroquine (250 mg daily). She has otherwise recently been in good health, and there was no past history of illness, including rheumatism and fever.

A biopsy from the knee showed similar changes to those of Patient III–1. A somewhat dense infiltrate was present at the junction of the lower dermis with the fat (Fig. 8), but no signs of fat necrosis or lipophagic granuloma were found.

A third sister (II–3) has never had subcutaneous nodules on the arms, legs and buttocks. However, she has at times conspicuous nodules over the second interphalangeal joints, with redness of the hands and poikilodermatous skin over the elbows. She has had somewhat painful palmar nodules on the right hand. No other members of this family had lesions in this distribution. She has had nodules on the hands since High School. Biopsies were taken of her elbows and fingers. A perivascular lymphocytic infiltrate surrounding the upper and mid-dermal vessels was the only significant histological finding.

Her daughter, aged 2 (III–5), has prominent nodules over nearly all the interphalangeal joints, and also on the elbows. These nodules were noted at birth. A biopsy specimen, taken from the right index finger, showed throughout all levels of the dermis a patchy and diffusely distributed lympho-histiocytic inflammatory cell infiltrate, localized around capillaries and eccrine sweat glands. The involved capillaries showed slight endothelial swelling and thickening of the walls. A small amount of thready basophilic granular material, probably mucus, separated the otherwise intact collagen bundles.

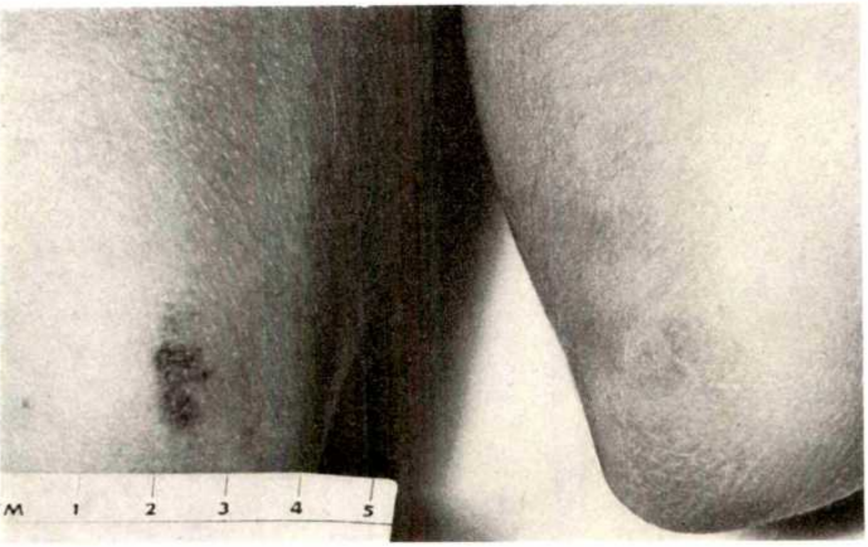

FIG. 5.—Nodules of elbow, one of which is purpuric following a minor injury (Case III–1).

Her son, aged 1 (III–6), developed in July 1970 a patchy erythematous scaly rash on the left cheek, which spread in a butterfly pattern to the other cheek (Fig. 9). It was refractory to hydrocortisone cream. Because of his age, this patient's blood was not examined at that time. However, because of the subsequent concern of the paediatrician about the rash on the face, then typical of discoid L.E., numerous tests were performed; these were normal (WBC, antinuclear antibody L.E. clot test, and haemoglobin). The rash subsided with the onset of winter. At the wish of the parents no biopsy was taken. No chloroquine was given because of its reported toxicity in small children. There are no nodules, so far, over the joints, and no systemic manifestations.

Multiple immunological studies were performed on this large family at the University of California Medical Center, with the results shown in Table I.

Both mothers with nodules on their legs and arms had antinuclear factor. During the summer one mother had a slightly positive L.E. factor and rheumatoid factor. These both subsequently reverted to negative.

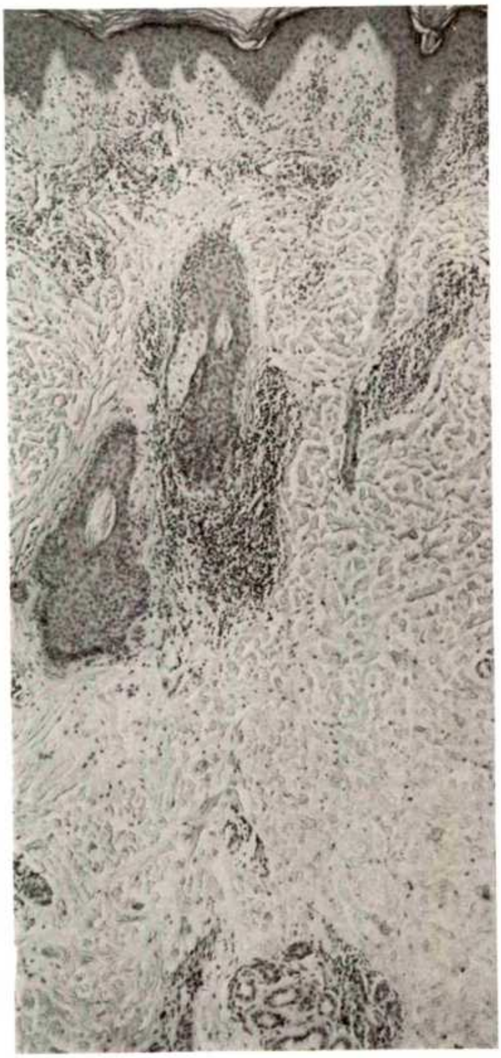

FIG. 6.—Biopsy of nodule of elbow, showing a perivascular lymphocytic infiltrate at various levels in the dermis (Case III–1). (H. and E. ×40.)

The grandmother with rheumatoid arthritis has rheumatoid factor and antinuclear factor, despite steroid suppression. However, one child (III–4), with no clinical signs, has an antinuclear factor like her mother. There have been reports of patients with sensitivity to autogenous DNA, accompanied by painful swelling and ecchymoses of forearms, legs and hands, similar to L.E. panniculitis, with complete suppression of the lesions by chloroquine (Levin and Pinkus, 1961; Schwartz *et al.*, 1962). However, all of our patients had negative anti-DNA studies. They were not skin-tested for DNA sensitivity because clinically they did not resemble the patients with this abnormality.

DISCUSSION

A familial incidence of lupus erythematosus and related disorders is well known (Beckett and Lewis, 1959; Grupper, 1963; Burch and Rowell, 1963, 1965; Reed *et al.*, 1967). Two of the 6 patients with L.E. profundus reported by Tuffanelli (1971) were sisters. Two siblings of Tuffanelli's third patient with L.E. profundus also had L.E. (one had discoid L.E. and the other systemic L.E.), and all her children had serological evidence of immunological abnormalities. Our patients were somewhat different clinically from the usual pattern of L.E. profundus as described by Irgang (1940), Arnold (1948), Fountain (1968) and Tuffanelli (1971) in that the onset was shortly after birth, and that the lesions showed a predilection for the tissues overlying the bony prominences of the limbs. Furthermore, the histopathology was different. In none of the biopsies was evidence found of marked necrotizing or fibrinoid changes in the fat, as is characteristic of L.E. profundus; nor were confirmatory histological changes of L.E., such as liquifaction degeneration, in evidence. The main histological change in our patients' nodules was that of a deep extension of lymphocytic vasculitis and mucin accumulation was usually an associated feature. This increase in mucin in some of the nodules may be of significance, as mucin accumulation is sometimes a feature in L.E. (Gold, 1954). The absence of any histological evidence of a palisading granuloma in our patients' nodules indicates that they are not rheumatoid in nature.

However, the following features may indicate that the family we report has indeed a unique variant of L.E. The proband at one time was found to have positive L.E. and rheumatoid factors, and one child in the family had developed a "butterfly" rash on the face, clinically suggestive of L.E. The rapid response of the nodules to chloroquine is also in keeping with this diagnosis. Several patients in the family were shown to have antinuclear factors.

Kaposi (1872) described transient nodules over the knuckles, elbows and knees in severely ill patients with probable systemic L.E. Kaposi (1883) may also deserve credit for having described L.E. profundus, but full details were lacking. Rheumatoid-like nodules have been reported in about 5–10% of patients with systemic L.E. (Tuffanelli and Dubois, 1964; Harvey *et al.*, 1954). An additional factor in the evaluation of this problem is the occurrence of positive L.E. tests in patients described as having rheumatoid arthritis with nodules (Allison and Bettley, 1957; Friedman *et al.*, 1957). However, the nodules in Allison and Bettley's patients showed rheumatoid histological changes. The biopsies of nodules occurring in systemic L.E. were reported by Larsen (1961) as showing non-specific inflammatory changes, or features of erythema nodosum and fat necrosis. Palisading granulomata were not encountered.

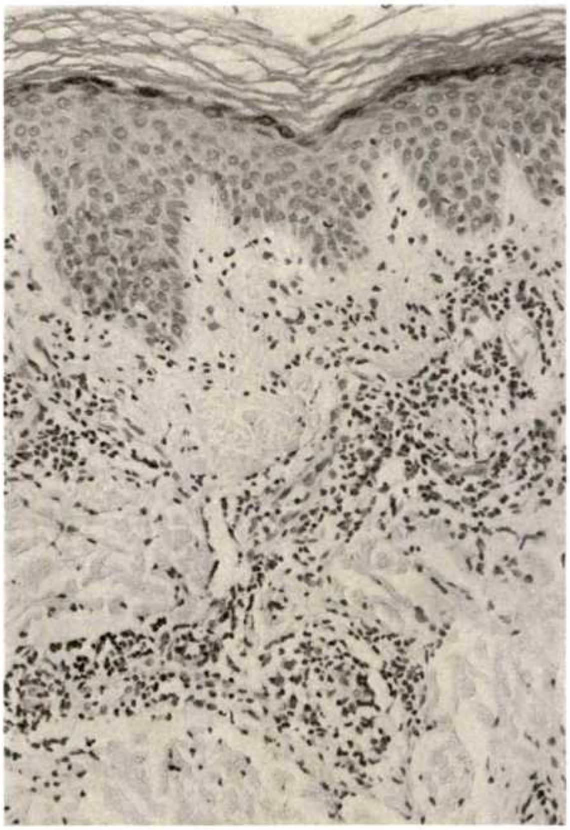

FIG. 7.—Detail of Fig. 6, showing the dilated papillary vessels loosely cuffed by small lymphocytes. (H. and E. × 100.)

It is notable that none of the patients in these reports of nodules associated with L.E. was exactly similar to our patients. This family appears to be unique in regard to the combination of an early age of onset, the familial incidence and the unusual distribution of the lesions. Additional aspects were the aggravation by sunlight and the rapid resolution with chloroquine therapy. In conclusion, this family represents either an unusual familial form of a collagen disease or a new genodermatosis with L.E.-like features.

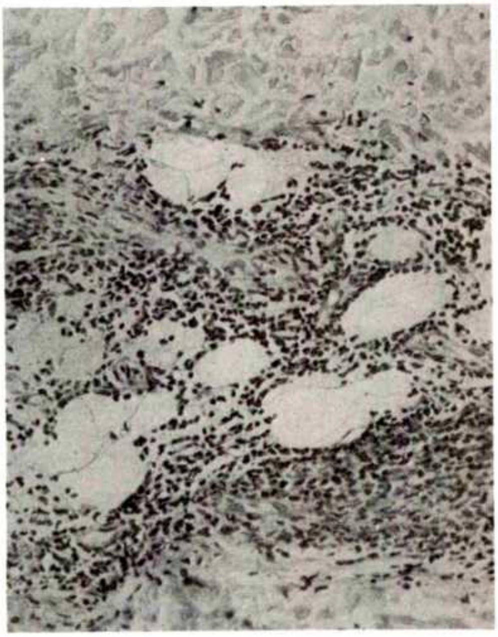

Fig. 8.—Biopsy of knee, showing a heavy lymphocytic infiltrate at the lower border of the dermis and extending into the fat.

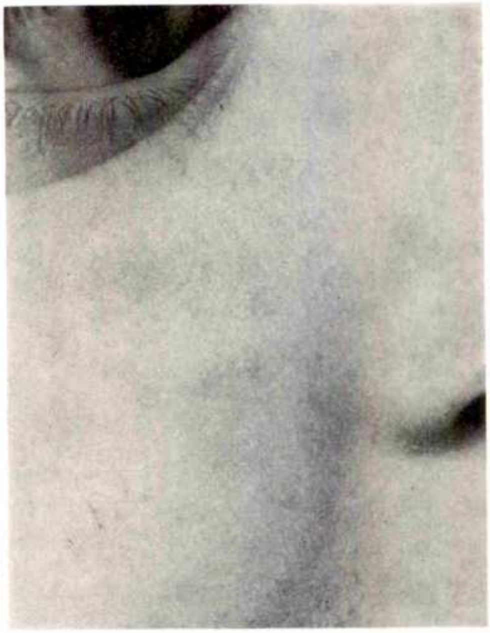

Fig. 9.—Scaly erythematous eruption of cheek, suggestive of discoid L.E. (Case III–6).

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ATYPICAL ERYTHROKERATODERMA WITH DEAFNESS, PHYSICAL RETARDATION AND PERIPHERAL NEUROPATHY

J. MARTIN BEARE, N. C. NEVIN, P. FROGGATT, D. C. KERNOHAN AND INGRID V. ALLEN

Summary.—An atypical form of erythrokeratoderma variabilis, profound bilateral perceptive deafness, physical retardation, and specific neurological disturbances are described in a girl aged 8 from County Tyrone, Northern Ireland. The differential diagnosis is discussed and abnormal EMG, nerve conduction and muscle biopsy findings are described. The syndrome is considered to be due to a " dominant " mutant gene. Only one case with a similar constellation of findings has previously been described (Schnyder *et al.*, 1968), although there are some differences in detail between the two.

ERYTHROKERATODERMA VARIABILIS, a rare genodermatosis, usually develops during the first year of life, and is characterized by capriciously outlined, fluctuating, erythrodermic and hyperkeratotic lesions as the only anomalies. This paper describes a syndrome manifesting an atypical form of erythrokeratoderma, with profound deafness, physical retardation and specific neurological disturbances. Only one patient with these general stigmata has previously been reported (Schnyder *et al.*, 1968), but the present case shows certain differences in detail.

CASE REPORT

A young girl aged 8 (date of birth: September 6th, 1963) was seen initially when aged 9 months, with an erythematous rash on the face, limbs and buttocks, first noticed at the age of one month, but possibly congenital. The mother's pregnancy and the birth and post-natal health of the child were unremarkable; birth weight was 4318 g and there is no maternal history of insult by known teratogens. On admission to hospital (May 1964) the skin lesions were seen to consist of erythematous patches with bizarre outline, bilateral but not quite symmetrical, and most marked on the face (Fig. 1), but affecting also the limbs and buttocks. The skin elsewhere was dry and scaly and there was no history of bullae, vesiculation, or pruritus. The margins of the erythematous patches changed only slowly over the next year of periodic in-patient observation.

The child was re-admitted for full investigation in January 1969, since when she has been under continual surveillance. The erythematous patches are now more extensive, involving the cheeks, eyelids and chin (Fig. 2), wrists, elbows, knees, popliteal fossae and particularly the buttocks (Fig. 3). The dorsa of the feet, however, are now the only areas with hyperkeratotic lesions. Scaling, pruritus and vesiculation are still absent. The lesions are unaffected by seasonal, physical or psychological factors, or by topical corticosteroids. The fingers and toe-nails show dystrophic changes, being thickened, irregular and yellow. The oral mucous membrane, particularly that of the gingivae and

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the alveolar ridges, show extensive areas of erythema. The lip commissures, which are constantly moist and raw, are severely scarred, due to chronic inflammation. The buccal aspect of the left angle of the mouth shows a raised white patch running along the occlusal line to the molar teeth, while, on the right side, there was a white pedunculated mass; this was excised for histology (see below). The dorsum of the tongue is deeply fissured, and the surface of the palate and the floor of the mouth are mottled and erythematous. Tooth complement, and dentition on radiological investigation, are normal in all respects.

Deafness, presumptively " congenital " (she allegedly never heard and has no speech), is perceptive and is clinically profound; the audiogram (March 21st, 1969) shows bilateral generalized loss, with minimal residual low-tone retention in both ears and slight middle-tone retention in the right (Fig. 4). The electroencephalogram (EEG) shows a dominant theta activity at 4–6 Hz, with slower waves at 3 Hz recorded from the post-central areas and occasionally associated with indefinite sharp waves. No focus or asymmetry was noted. The cause of this mild abnormality is unexplained.

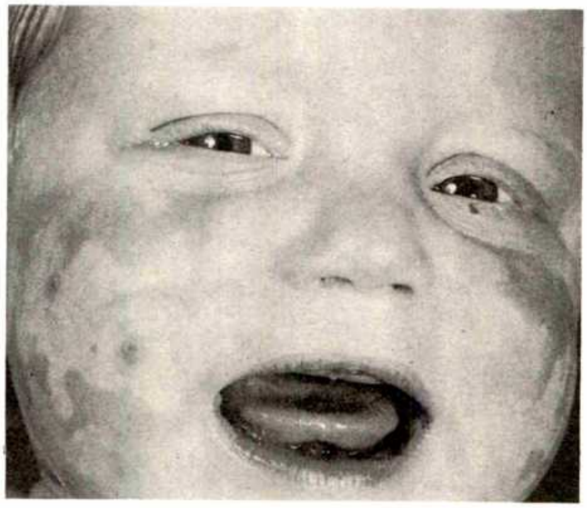

FIG. 1.—Patient aged 9 months. Bilateral erythematous areas, with bizarre outline on the cheeks.

Developmental progress has been as follows: sitting-up at 6 months, crawling at 7 months, pulling herself up at 9 months, and walking at 20 months. At 26 months she was noticed to be walking on her toes: this progressed until, by early 1970, when aged 6½ years, she could no longer put her heels on the ground and walked stiffly on her toes, with knees flexed. Neurological examination of the lower limbs revealed absence of all tendon reflexes. On April 15th, 1970 she had a bilateral lengthening of the Achilles tendon, with a satisfactory result. At 26 months she was also thought to be mentally retarded: I.Q. 67 (Terman-Merrill revision form L) was considered to be an nderestimate because of lack of co-operation. Subsequent development supports this view, and she now (October 1971) scores normally for her age on WISC performance. Although birthweight was satisfactory, the patient's height and weight have subsequently remained below the 3rd centile. In June 1971 her height was 112 cm and weight 16·6 kg. Bone age was initially retarded—at age 5½ years it was estimated (wrist x-ray) to be less than 4 years; but, on re-examination (October 1971), it was consistent with calendar age, although the carpal bones were considered to be small.

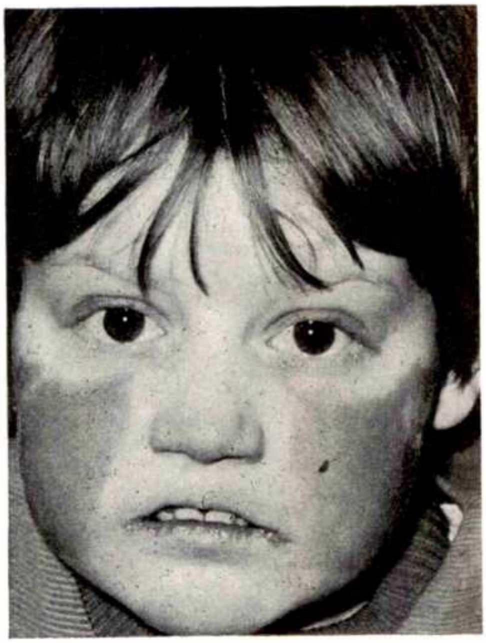

FIG. 2.—Patient aged 5 years 5 months. Erythematous areas, now pronounced, involving cheeks, eyelids and chin.

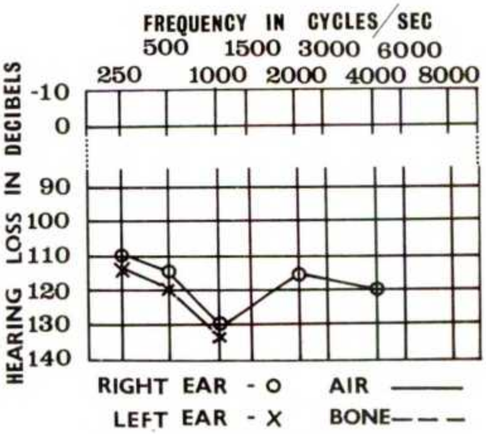

FIG. 4.—Audiogram demonstrating bilateral generalized loss, with residual low-tone retention in both ears, and slight middle-tone retention in the right.

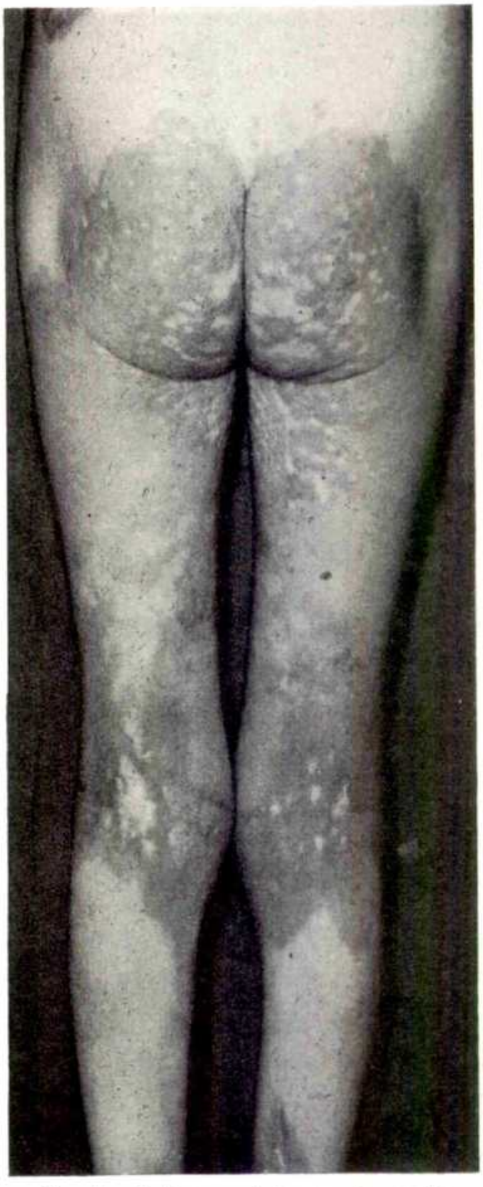

FIG. 3.—Patient aged 5 years 5 months. Erythematous areas, involving buttocks, thighs and popliteal fossae.

Electromyogram (EMG) and motor nerve conduction are both abnormal. EMG (right quadriceps) shows no spontaneous activity, except for occasional fibrillations. The muscle action potential has a mean duration of 15·9 m/seconds and a mean voltage of 3 mV. There are 4% polyphasics. Nerve conduction studies (right median nerve) show a motor conduction velocity of 35 m/seconds, substantially less than normal. Both EMG and conduction studies indicate a neuropathy.

The patient is seventh of a sibship of 9; none of her sibs manifests any of the stigmata. The audiogram of parents and sibs is abnormal only for the father, who has a mild high-tone deafness (R = 30+, L = 15+ decibel loss at 8000 Hz). The father was aged 37 and the mother 33 at the birth of the patient, and they deny blood relationship. Other than the proposita there is no member of either parent's family with relevant skin, auditory or neurological abnormality.

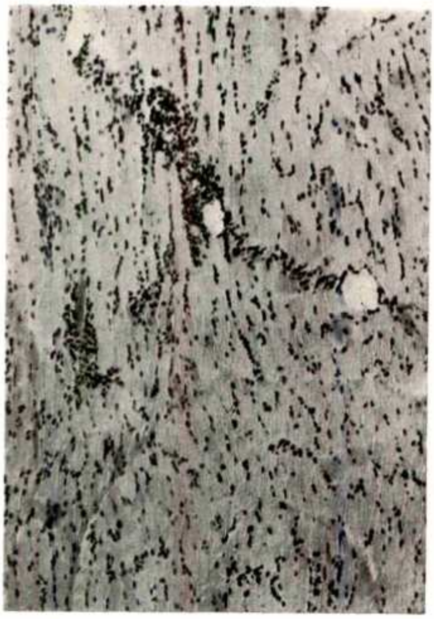

FIG. 5.—Longitudinal section of gastrocnemius, illustrating focal infiltration of lymphocytes around isolated muscle fibres apparently undergoing dissolution. (H. and E. × 90.)

Laboratory investigations—At age 7 years these showed: Hb 12·0 g/100 ml; total w.b.c. 10,000/mm^{-3}, with a normal differential count; WR, Kahn, and RPCFT all negative; serum calcium and phosphorus respectively 9·6 and 5·5 mg/100 ml; blood urea 31 mg/100 ml; serum alkaline phosphatase 28 units; serum transaminases (SGOT and SGPT) respectively 44 and 16 units; serum vitamin A 69 μg/100 ml; serum creatinine phosphokinase 16·72 mU/ml. Plasma proteins were (g/100 ml): total 6·7; albumin 2·9; α_1-globulin 0·3; α_2-globulin 0·9; β-globulin 0·9; γ-globulin 1·7. Serum immunoglobulins, estimated by simple radial diffusion in agar, were (mg/100 ml): IgG 1450; IgA 540; IgM 86. Karyotype is of a normal female (46, XX); urinary amino-acid chromatography was normal. All these findings are considered unremarkable.

Histology.—Biopsy sections of a florid skin lesion show marked hyperkeratosis of the epidermis and a mild chronic inflammatory infiltration around the blood vessels of the upper dermis. The oral mucosa shows marked intra-epithelial oedema, extending from the basal layer to the surface, where it has produced a basket-weave appearance. The dermis shows no special features. Four deciduous teeth were examined. Caries was marked and had spread rapidly to involve the pulp, where changes varied from acute inflammation to necrosis. Secondary dentine response was slight.

The biopsy of the gastrocnemius, taken before operative elongation of the Achilles tendon (April 1970), is abnormal: there is little variation in fibre size and they are generally of small diameter (fibre diameter range 8–33 μm; mean 20 μm). There is some generalized atrophy of groups of fibres, and some thin basophilic " regenerating " fibres are present. There is no evidence of abnormal glycogen or lipid storage. The most striking change in longitudinal section is focal infiltration of lymphocytes around isolated fibres apparently undergoing dissolution (Fig. 5). Associated with the atrophy there is a slight increase in fat and fibrous tissue (Fig. 6), but the blood vessels are normal. On *electronmicroscopy* the picture is essentially normal, although some myofibrils are split and show focal degeneration with Z band dissolution. In these areas an increase in mitochondria is noted, and the sarcoplasmic reticulum is prominent. In general the myofibrils are small but uniform and show only mild focal degenerative changes. These findings are in keeping with neurogenic muscle atrophy. However, the marked inflammatory infiltration, with isolated fibre dissolution, although observed in the early stages of neurogenic atrophy, could also indicate a primary muscle disorder associated with the peripheral nerve abnormality.

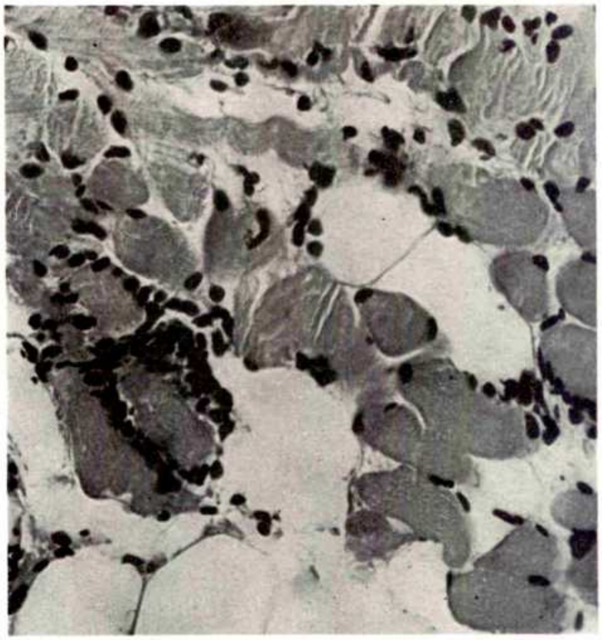

FIG. 6.—Atrophic changes in the muscle fibres, with a slight increase in fat and fibrous tissue. (H. and E. ×300.)

DISCUSSION

Erythrokeratoderma variabilis was first described by Mendes da Costa (1925)—in a mother and daughter—and is now a well-recognized although rare condition (e.g. Noordhoek, 1950; Coles, 1954; Schnyder and Sommacal-Schopf, 1957;

Sommacal-Schopf and Schnyder, 1957; Wells, 1962; Brown and Kierland, 1966; Schellander and Fritsch, 1969; Cram, 1970). It has 2 distinct components: erythema and hyperkeratosis. The former consists of capriciously outlined, ubiquitous, erythematous patches which alter in size and shape from day-to-day, or even from hour-to-hour. They are macular and exhibit no secondary changes such as scaling. The hyperkeratotic patches, which are usually persistent, also have a striking geographic outline; but, unlike the erythematous patches, they tend to be site-specific, involving particularly the face and buttocks and the extensor surfaces of the limbs. Affected subjects rarely show associated defects or constitutional disturbances (Brown and Kierland, 1966).

We consider that the case presented in this paper is an example of erythrokeratoderma. Clinically the skin lesions are typical, except that, in the last 3 years, close scrutiny has failed to show the marked fluctuation in size, location and shape such as are classically described in erythrokeratoderma and, as this patient showed earlier, though only slightly. We have no explanation for this phenomenon.

Other erythrokeratodermas to be considered, but which on clinical grounds are less likely, include the *genodermatose en cocardes* (Degos *et al.*, 1947), erythema gyratum perstans (Fox, 1881), erythema annulare centrifugum (Darier, 1916–17), erythema figuratum perstans (Wende, 1906, 1908), erythema gyratum repens (Gammel, 1952), erythema exsudativum perstans (Sachs, 1901), and familial annular erythema (Beare *et al.*, 1966). Pertinent stigmata of these and other relevant erythemas are tabulated by Beare *et al.* (1966).

This case, however, has a number of features additional to erythrokeratoderma. Physical growth is retarded (stature and weight below the third centile), there is bilateral profound perceptive deafness, presumptively from birth, and a bilateral talipes deformity, now shown to be a neuropathy. These may be chance findings or, alternatively, stigmata comprising a distinct entity which must be distinguished from others with a similar aggregation of findings. These are: Refsum's syndrome (Refsum, 1945)—but here the skin lesion is an ichthyosis and the deafness, which is less marked, is not congenital; familial amyloidosis (Muckle and Wells, 1962)—but this can easily be distinguished on clinical grounds; and the case, already mentioned, of Schnyder *et al.* (1968). In the last-named the erythrokeratoderma was also " atypical " and associated with physical retardation, but the deafness was unilateral (the audiogram showed substantial hearing retention in the right ear) and, although the patient had a bilateral talipes deformity, unlike our case, EMG, muscle biopsy and nerve conduction patterns were described as normal. Furthermore, in the present case the serum vitamin A level was normal, whereas in that of Schnyder *et al.* (1968) it was reduced. Nevertheless, the similarity between the 2 patients is striking and suggests that they may be of a similar genotype, the differences in the clinical presentation being due to minor differences in phenotype expression.

Erythrokeratoderma variabilis often aggregates in families, when it is seemingly due to an autosomal dominant gene (e.g. Noordhoek, 1950; Schnyder and Sommacal-Schopf, 1957), although a number of sporadic cases have been reported. In neither the present family nor that of Schnyder *et al.* (1968) was any other affected individual identified, and the most likely explanation is that both propositae are expressions of distinct and possibly identical dominant mutant genes, different from those causing other erythrokeratodermas.

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PERIORAL DERMATITIS: AETIOLOGY AND TREATMENT WITH TETRACYCLINE

ANGUS MACDONALD AND MICHAEL FEIWEL

Summary.—" Perioral dermatitis " has been studied in 29 patients; all were females in their child-bearing years, with ages ranging from 18 to 39 (mean 27).

Twenty patients showed a marked flushing tendency, but the condition was apparently unrelated to other factors studied, including colouring, skin type, pregnancy and the contraceptive pill.

Perioral dermatitis is a distinct entity and not a variant of seborrhoeic dermatitis or rosacea. All patients were greatly improved by tetracycline therapy for 2-3 months, after which relapse was unusual.

" PERIORAL DERMATITIS " (Hjorth *et al.*, 1968; Finlay, 1969; Dijk *et al.*, 1970) occurs in young women as a papular erythema varying in extent from a few inconspicuous lesions to a disfiguring eruption involving most of the area bordered by the nasolabial folds and the sides of the chin. The well-delineated clinical picture and the age and sex distribution would suggest that " perioral dermatitis " is a distinct clinical entity; however, some regard it as a variant either of seborrhoeic dermatitis or of rosacea. Again, while a few dermatologists claim they have always been aware of the condition, most consider that the clinical picture is one which has appeared only recently.

Little is known of the aetiology. Early reports (Frumess and Lewis, 1957) considered that light sensitivity might be a factor. More recent publications have been less definite but have offered no alternative explanation. As regards histopathology, Marks and Black (1971) showed that the changes differed significantly from those found in rosacea and were suggestive of an exogenous cause (although of unspecified nature). Until recently treatment was regarded as unsatisfactory; most patients were given various topical steroid preparations, without much effect. During the last 2 or 3 years, however, the efficacy of oral tetracycline has become recognized. This therapy is now regarded in the United Kingdom as being fairly specific.

We report observations on 29 patients with perioral dermatitis. The study was made so as to throw light on possible aetiological factors and to evaluate the results of treatment.

PATIENTS AND METHODS

Because of their rash the patients had been referred by their general practitioners to the Dermatology Out-patient Clinic. The criterion for inclusion in the study was a diagnosis of " perioral dermatitis ", based on the clinical appearances already described and a duration of the rash of 3 months or more. The findings were recorded on a proforma and, in addition to a description of the rash, these included age, sex and occupation of the patient, family history, details of previous therapy (including the

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taking of the contraceptive pill), hair and eye colouring, "skin-type" and presence of any ocular signs and symptoms. Note was taken if the patients exhibited a flushing tendency and of precipitating factors when relevant.

Skin type.—This was assessed by dividing patients into 4 categories according to their reaction to sunlight exposure: +++ denoted immediate tanning (i.e. after 24–48 hours exposure); ++ indicated tanning after an average period of time; + meant that patients tanned only after several weeks exposure; 0 was given to those who never tanned at all.

Eye colour was recorded as black or almost black, blue and other.

Hair colour was similarly noted as black or almost so, blond and other.

Flushing tendency.—This factor proved difficult to evaluate. Various methods were tried in an attempt to measure objectively the size and degree of a flush induced by drugs, alcohol, heat, etc., but these gave inconclusive and variable results and were abandoned. Patients were therefore assessed by being asked whether they considered themselves more prone to flush or blush than friends or relatives. If they replied with an affirmative and, at the same time, exhibited a vigorous, visible flush, this was then recorded.

Ocular manifestations.—The patients' eyes were examined with a Haag Street Slitlamp. The presence of vascular engorgement of conjunctival and limbal blood vessels and/or corneal opacities was looked for particularly.

Controls.—For all the above observations controls were obtained from an age- and sex-matched population of other patients who were attending the Skin Department and who were suffering mainly from virus warts.

Treatment.—The patients were instructed to apply topical ½% or ¼% ichthyol in water, or ½% or 1% sulphur in calamine lotion at night, alternating with 1% hydrocortisone cream by day.

If there was no favourable response after 1 or 2 weeks, tetracycline tablets were prescribed in a dose of 250 mg 3 times daily for 1 week and, thereafter, twice daily. The tablets were to be taken with water and on an empty stomach; and, in view of the sex and age of the patients, it was emphasized that they were to be discontinued if they became pregnant and were not to be taken during breast feeding. The patients were seen regularly in the Out-patient Clinic either to the point of cure and discharge or to loss from follow-up due to failed attendance. The defaulted patients were surveyed by post in an attempt to assess duration of benefit from treatment and their current condition.

RESULTS

The 29 patients have been observed over a period of 3 years.

Signs and symptoms.—The morphological features of the eruption conformed to the previous descriptions, and usually comprised small papules of less than 2 mm diameter on a background of erythema in the perioral distribution. The mean duration of symptoms prior to referral was 23 months, the range being from 3 months to 7 years. Most patients had been treated with several of a large variety of agents, usually without benefit. As regards the potent topical (fluorinated) steroids, these had been used by 26 patients. Three had been improved, 8 were made worse and 13 remained fairly static. By this, patients usually denoted that, while the cream was being applied regularly, the skin condition was practically suppressed, but that it deteriorated whenever frequency of applications was reduced.

Eye signs.—The first 15 patients were examined with the slit lamp; no abnormality was found. Routine, but less searching, examination of subsequent patients also revealed no ocular features.

Table 1

	Tanning			Hair Colour			Eye Colour		
		Nos.	%		Nos.	%		Nos.	%
Patients	+ + +	3	10	Blond	6	21	Blue	14	48
	+ +	8	28	Black, or almost	12	41	Black, or almost	2	7
	+	14	48	Other	11	38	Other	13	45
	0	4	14						
	Totals	29	100						
Controls	+ + +	9	31	Blond	6	21	Blue	16	55
	+ +	4	14	Black, or almost	8	28	Black, or almost	4	14
	+	11	38	Other	15	51	Other	9	31
	0	5	17						
	Totals	29	100						

Sex.—All patients were female.

Age.—Mean age was 27 years, with a range of 18–39 years.

Contraceptive pill.—Fourteen of 29 patients were taking various different makes of " the pill " when symptoms developed; 7 were still using it regularly when they presented with the rash, and a few had started on " the pill " after onset of the eruption. No relationship, therefore, emerged between " the pill " and the skin condition.

Pregnancy.—Two patients developed the rash when pregnant; 2 others, however, who became pregnant after benefiting from treatment, did not relapse.

" *Skin Type* ", *eye and hair colouring.*—No differences were found between the patients and the control group (Table I).

Flushing.—This was a prominent feature in 20 patients (69%) and in 7 (26%) of the control group ($P < 0\cdot001$). Most patients who flushed severely indicated that they had done so all their life, or since early teenage.

Response to treatment.—Only 4 patients benefited by local therapy alone. The remainder, who then received tetracycline tablets, all responded well.

Improvement was usually apparent within 10–14 days of starting tetracyclines, and the mean duration of treatment was 2–3 months. However, after their loss to follow-up, some patients may have continued to take tetracycline prescribed by their own doctors so that treatment could have been more prolonged. Twenty-three patients had discontinued regular attendance when the survey was concluded. Four were known to have left the country. Thirteen of the remaining 19 replied to the postal request for follow-up information; all considered themselves cured or considerably improved, a few occasionally using local therapy. Two patients who still attend the clinic have been taking tetracyclines continuously for 2 years. They relapse whenever they try to stop treatment. A number of others suffered recurrence in the early stages of their dermatitis when they discontinued their tablets on their own. This usually occurred when they did so after less than 4 weeks of therapy. If treatment was continued for a longer period, the tendency to relapse seemed to diminish.

DISCUSSION

The only factor relevant to the aetiology of " perioral dermatitis " that emerged from our study was predisposition of the affected group to flush excessively, as compared with the controls. The relationship, however, between such vasomotor instability and pathogenesis of " perioral dermatitis " requires further elucidation. Other conditions characterized by diffuse erythema, such as rosacea or " steroid acies ", appear also to be related to a flushing tendency. In rosacea the connection between eye signs and a flushing predisposition was established by Starr and Macdonald (1969), but no ocular findings were observed in the group of patients with " perioral dermatitis ". The dermatitis is seen only in women who are in their potentially reproductive phase, but it is not associated with the taking of the contraceptive pill. There is, therefore, another factor as yet unidentified, peculiar to this group of women. Clear-cut potential exogenous allergens such as make-up have been excluded in previous studies, but there may be more subtle ones. An early impression that fair-skinned women who would not tan easily were particularly prone to " perioral dermatitis " suggested that sunlight sensitivity might be aetiologically important. However, this turned out to be merely a reflection of the high population in our area of patients of " Celtic " origin, for many of our controls had similar colouring and inability to pigment readily. " Perioral dermatitis " is not a variant of seborrhoeic dermatitis, as evidenced by the exclusive female sex incidence, absence of seborrhoeic dermatitis elsewhere, lack of improvement with topical steroids, and good results with tetracycline. It shares with rosacea a response to tetracycline and the predisposition of affected patients to easy flushing, but it has a different histology (Marks and Black, 1971) and no ocular manifestations. Relationship of " perioral dermatitis " to use of potent topical steroids needs to be clarified; their effect on papules is variable, but they appear to aggravate the erythema. A recent report (Weber, 1972) attributes this condition entirely to the application of these medicaments. This author, however, uses a much less strict clinical definition than has been adopted in this paper and in earlier publications. The dermatoses which he groups together under the title of " rosacea-like dermatosis " are more closely akin to the by now well known "steroid facies" (Macdonald, 1971) or even "tinea incognito" (Ive and Marks, 1968) than to " perioral dermatitis ". In fact, none of the patients which he describes and illustrates would be diagnosed by us as having " perioral dermatitis ". Most of our patients had received steroids topically, but they maintained that their rash had been similar before they started this treatment. Surprisingly, Marks and Black (1971) appear to have taken no account of the possible effect of such steroids on the histopathology of the lesions making interpretation of their findings difficult. " Perioral dermatitis " is probably a " new " condition; it certainly has a distinctive clinical picture, a striking sex and age distribution and a natural history which is usually self-limiting when tetracycline treatment is employed.

The success of tetracyclines may lie in the effect on the microflora in and around hair follicles, but " perioral dermatitis " has little resemblance to acne occurring at similar sites, or even less to a staphylococcal folliculitis. No double-blind trial of tetracyclines in this condition has yet been reported; therefore our claim of its value (and its widespread use) requires such confirmation. Nevertheless, we have shown that many patients can discontinue tetracyclines after a course of some 2 or 3 months, without relapse.

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NICKEL ANTIBODIES*

LOUIS FORMAN AND SUZANNE ALEXANDER

Summary.—Eighteen patients with nickel sensitivity and 12 control patients were examined. Lymphocytes from these patients were cultured together with nickel sulphate (antigen). Evidence of cellular hypersensitivity was obtained by the following procedures:

(1) Estimation of the percentage of lymphocytes transformed to lymphoblasts; (2) the uptake of C^{14} thymidine in the lymphocyte cultures as evidence of DNA synthesis in the " challenged " cells; (3) the macrophage migration inhibition test, using the supernatant fluid from the cultures and guinea-pig macrophages.

FORMAN, in his Watson-Smith oration (1961), stated that, by adding 0·1% nickel sulphate solution to the serum of nickel-sensitive patients, a band of precipitation was produced in these sera and that this was significantly wider than in the normal control sera.

An attempt to determine the presence of serum antibodies by the methods available at that time, e.g. complement fixation (reported positive by Wendleberger and Fröhlich, 1954) Rose Waaler, Coombs or Latex precipitation tests on the sera of 25 nickel-sensitive patients gave negative results. This observation prompted the use of the lymphocyte transformation tests, when these became current, as an *in vitro* system to demonstrate nickel sensitivity. Later, when macrophage migration tests were available, these were used as yet another parameter of demonstrating nickel sensitivity.

MATERIAL AND METHODS

Clinical Material

Thirty patients were tested. There were 18 patients with known nickel sensitivity and 12 " control " patients who had no known nickel sensitivity.

Rationale of Tests

The lymphocyte transformation test depends upon the ability of lymphocytes from a sensitized patient to transform into blast cells (Fig. 1, 2) *in vitro* when in contact with the sensitizing substance; during this transformation, synthesis of nuclear protein can be estimated by the uptake of C^{14} thymidine, and further during this transformation various " lymphokines " are liberated which inhibit, temporarily, the migration of macrophages, which normally move outwards from a capillary tube to engulf foreign particles. The area of inhibition when the macrophages are in contact with " lymphokines " is measured with a planimeter and compared with the surface covered by macrophages not so exposed. This forms the basis of the macrophage migration inhibition test (MIF).

Method of Lymphocyte Culture

Twenty ml of blood were collected into heparinized universal containers, and 10 ml of blood into a plain tube. One part 3% dextran was added to 3 parts of blood. Five

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* Based on a paper presented to the British Association of Dermatology, July 1970.

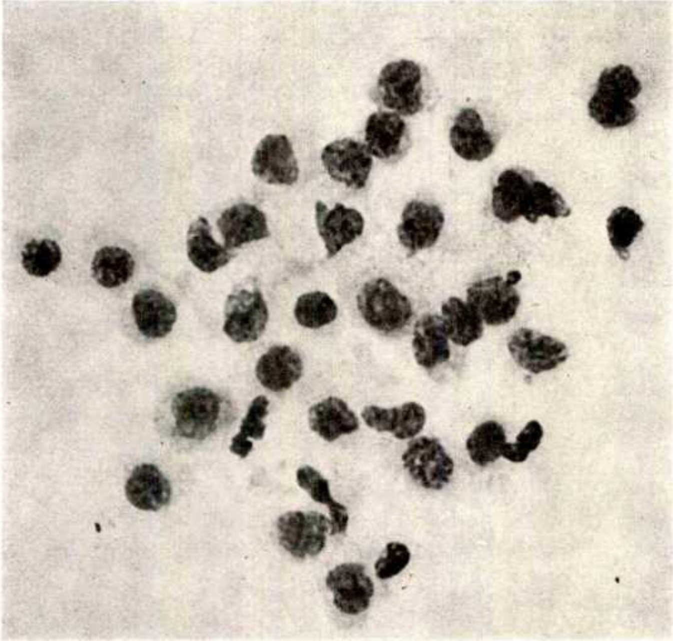

FIG. 1.—Lymphocyte stimulation with phytohaemagglutinin, showing blast cell response. (× 256)

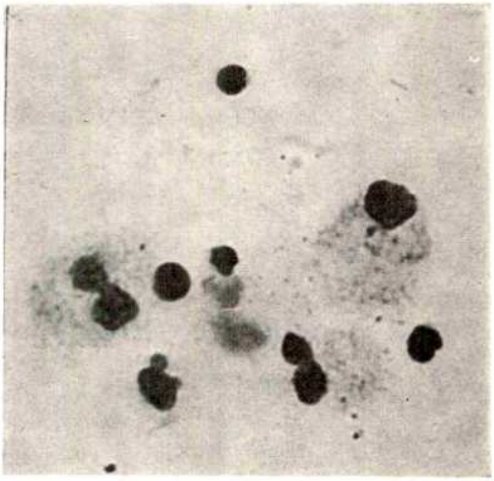

FIG. 2.—Lymphocyte stimulation with nickel sulphate. Some polymorphs and lymphocytes are present, as well as blast cells. The blast cells are smaller than those from PHA-stimulated cultures. (× 256)

ml aliquots of this mixture were put into sterile tubes and incubated at 37°C for one hour.

The supernatants (plasma and cells) were taken off into another sterile tube (3 supernatants per tube) and spun at 1200 rev/min for 10 min. The plasma was discarded and each pellet resuspended in 1 ml of TC 199; the volume was made up to 6 ml/tube. These were then spun at 1200 rev/min for 10 min and the supernatant again discarded.

The cells were made up to a volume of 3 ml (approximately 1 ml per tube); 0·1 ml of cells were taken and put into 0·8 ml of TC 199 and 0·1 ml Trypan blue and counted. They were diluted with TC 199 to give 10^6 cells/ml. Serum was added to give a final concentration of 10% in the diluted cells. One ml aliquots of these cell suspensions were then used, and 0·1 ml of antigen per tube added.

Phytohaemagglutinin (PHA) was diluted 1 in 10, in TC 199, and used in 0·1 ml aliquots. The tubes were gassed with a 5% CO_2/95% air mixture for a few seconds; the lids were then screwed on, and the tubes incubated for 3 days.

Late on the third day C^{14} thymidine was added (0·1 ml per culture—i.e. 0·25 μCi) and incubated overnight at 37°C. It was then spun at 1000 rev/min for 6 min. The supernatant was collected for macrophage migration inhibition tests (MIF). One ml of 10% normal saline at 4°C was added, whirlymixed to resuspend, and 1 ml of 10% trichloracetic acid (TCA) added, and mixed. It was allowed to stand for one hour at 4°C. The volume was made up to 10 ml per tube with 5% TCA and filtered through 2·5 cm diameter glass fibre filter pads. The tube was refilled with 5% TCA and poured through the filter, then washed with 2 × 10 ml of AR methanol. The pads were put into counting vials and dried at 60°C (without lids). To these dried pads 6 ml of toluene scintillation fluid was added. The scintillation fluid contained 5 ml of hydroxide of hyamine per litre. The disrupted pads were then counted in a Packard Tricarb liquid scintillation counter by standard methods.

For histological examination of the films, the cells were resuspended on the third day in 1 ml of normal saline; but, instead of C^{14} thymidine or trichloracetic acid, 5 ml of aceto-methanol (one part acid to 3 parts AR methanol) were added and the cells spun at 1000 rev/min for 5 min and resuspended in 5 ml of aceto-methanol. These tubes were allowed to stand at room temperature for half an hour and then spun at 1000 rev/min for 5 min and each pellet of cells was made up in 1 ml of aceto-methanol. Two smear preparations were made from each tube, air dried and stained with Jenner–Giemsa stain, and mounted for differential cell counting.

Method of Macrophage Migration Inhibition Tests

Young adult guinea-pigs of between 300 and 500 g body weight were injected intraperitoneally with 25 ml warmed sterile paraffin to induce a sterile peritonitis. The animals were sacrificed on the fourth day after injection and the peritoneal exudate collected, after the addition of 20 ml of culture medium to the peritoneum. The peritoneum was subsequently washed out with a further 30 ml of tissue culture medium (Hanks balanced salt medium + Tris buffer + 4 units of heparin/ml). The exudate was collected in siliconized glassware and spun at 800 rev/min for 5 min at 4°C, and then washed twice in Hanks Tris Medium. The cells were resuspended in 3 ml of TC 199 and counted, using 1 in 10 Trypan blue stain. The cells were then adjusted to 7 × 10^6 viable cells/ml in TC 199 + 10% heat-inactivated guinea-pig serum + 5% foetal calf serum at pH 7·4. Sealed siliconized capillary tubes were filled with this cell suspension, using a 5 inch spinal needle. The capillary tubes were centrifuged at 500 rev/min for 5 min inside sterile 6-inch tubes, the latter having cottonwool-plugged bottoms and capped tops. While the cells were spinning and washing, special culture plates were prepared, adding sterile silicone in small blobs to each chamber.

When the cells were spun down, the capillary tubes were cut on the cell side of the cell/liquid interphase, using a diamond pencil and heavy forceps. Care was taken not to handle the capillary tube at the cell end. With the help of fine forceps, the closed end of the capillary tubes were placed on the silicone blob, taking care not to get grease on the open end of the tube. Each tube was checked for firm adhesion, and the wells of the culture plates were filled with 0·5 ml of supernatant fluid from the lymphocyte cultures. The culture chambers were incubated at 37°C in a humid atmosphere and examined at 24 and 48 hours.

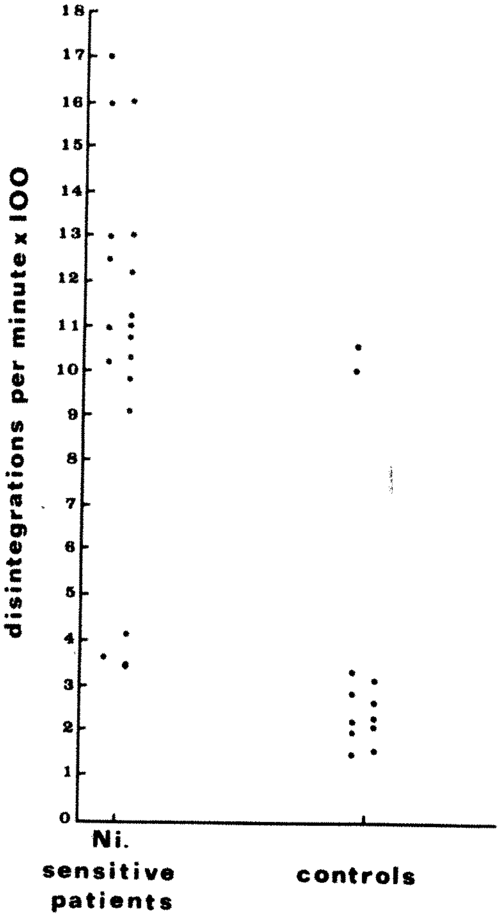

FIG. 3.—Results of C^{14} thymidine uptake in nickel-sensitive patients (left), and controls (right).

RESULTS

The lymphocyte transformation tests, as measured by C^{14} thymidine uptake and/or direct staining and counting of blast cells, were positive in 15 of 18 patients with alleged nickel sensitivity, and negative in 3 (Fig 3 and Table I).

Three patients who had been registered as nickel-sensitive on clinical findings, and who had had positive patch tests, but negative transformation tests, were recalled and re-tested. Two patients were found to be no longer nickel-sensitive clinically, and this was confirmed by patch testing with 2·5% nickel sulphate.

TABLE I.

Patient number	Disintegrations per min (corrected for quenching) after stimulation with nickel			Saline
	Control patients			
1	272	260		106
2	228	220		89
3	1080	1071		120
4	248	300		133
5	212	208		200
6	184	166		112
7	981	1004		96
8	296	280		87
9	126	120		64
10	140	144		98
11	328	342		160
12	351	331		145
	Nickel-sensitive patients			
1	961	981		80
2	341	369		81
3	1620	1660		163
4	1267	1241		137
5	389	371		33
6	1111	1009		46
7	1328	1270		49
8	1103	1098		29
9	1057	1031		44
10	913	903		605
11	1598	1632	1680	115
12	406	421	431	754
13	1116	1161		347
14	1005	1017		41
15	1765	1687	1799	120
16	1081	1076		105
17	1201	1239	1222	46
18	1320	1330		133

This confirms Morgan's view that a proportion of nickel-sensitive patients lose their sensitivity over a period of time. The third patient (Fig. 4), who was registered as nickel-sensitive and who had had a positive patch test 3 years previous to the lymphocyte stimulation tests, was recalled for further examination. He was found to have developed Hodgkin's disease. The patch test to 2·5% nickel sulphate was repeated and the result was negative. The negative patch test to nickel sulphate was obtained one month after the negative lymphocyte transformation test. The response of the lymphocytes to phytohaemagglutinin was also depressed (Fig. 3), and this decreased reactivity of the cells may have been due to either the patient's Hodgkin's disease, or the immuno-suppressive therapy he was receiving for its treatment.

Of the 12 control patients with no known nickel sensitivity, 2 gave positive results with lymphocyte transformation tests and 10 gave negative results.

On recall, the 2 patients who gave positive results stated that they knew they were sensitive to nickel. They had not previously stated this, as they had found avoiding nickel was adequate to keep them symptom-free!

Fig. 3 shows the nickel-sensitive patients' counts on the left and control counts on the right, and indicates that these do seem to represent 2 different groups of people.

Table II represents the lymphoblast transformation in patients and controls.

TABLE II.—*Percentage of Blast Cell Transformation after Incubation with Nickel*

Patient number	Nickel-sensitive patients	Control patients
1	16	3
2	12	4
3	25	17
4	20	3
5	12	4
6	18	2
7	18	8
8	14	4
9	16	3
10	8	4
11	22	2
12	3	3
13	16	
14	12	
15	26	
16	15	
17	16	
18	14	

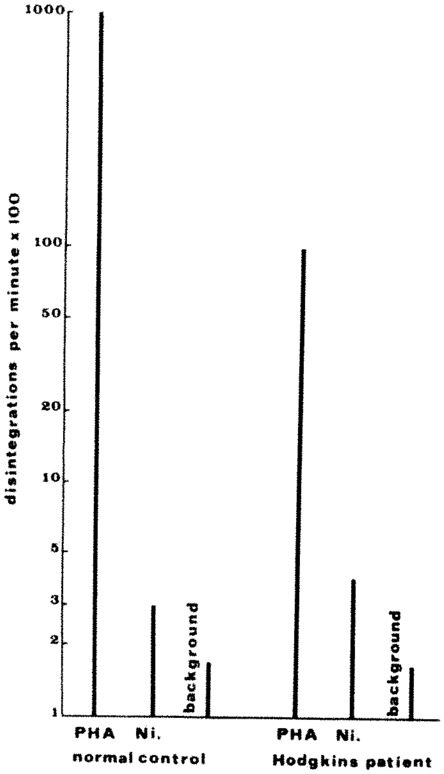

FIG. 4.—Difference in response of C^{14} thymidine uptake in a normal, non-nickel-sensitive patient (left) and a patient with Hodgkin's disease who had been nickel-sensitive in the past.

Autologous Plasma Factor

The importance of the presence of autologous plasma in the dynamics of the lymphocyte transformation test in nickel-sensitive individuals is indicated by the following experiment. Two of the cultures were repeated, using the nickel-sensitive patients' white cells, after washing and resuspension in Group O pooled plasma. No transformation took place. This would indicate that the autologous plasma was necessary, perhaps for the binding of the nickel, or by adding a substance to the cells necessary for transformation.

Macrophage Migration Inhibition Factor

This was found in the supernatant fluid of lymphocyte cultures of 3 patients with nickel sensitivity and in none of the controls. These findings confirm those of Macleod *et al.* (1970), but are not in agreement with Pappas *et al.* (1970), who found that nickel acetate was a non-specific stimulant. We used nickel sulphate instead of nickel acetate as an antigen, and this may account for the difference in our results.

CONCLUSION

The lymphocyte transformation test, estimated morphologically and by uptake of C^{14} thymidine, gave evidence that, in nickel-sensitive individuals, the lymphocytes were sensitized, and reacted when challenged with 2·5% nickel sulphate. A parallel demonstration of production of MIF from the supernatant of the stimulated cultures was demonstrated in 3 nickel-sensitive patients and none of the 6 controls.

Two patients who had been clinically and patch test positive 3 years before the above tests were made were found to be free of dermatitis and patch test negative on recall.

In practice, clinical observation and patch testing will detect the great majority of the sensitive individuals who present at the clinic. However, the patch test may at times be difficult to interpret and may itself cause a severe local reaction and even an exacerbation of an existing dermatitis. The *in vitro* tests described above may be of value under these circumstances.

This work was carried out in the Inter-Departmental Laboratory, Guy's Hospital, London SE1 9RT. S.A. is indebted to the Dunhill Fund for financial support. Our thanks are due to Mr P. Parkes for technical help.

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PILO-SEBACEOUS DUCT OBSTRUCTION AND ACNE

R. L. HOLMES, M. WILLIAMS AND W. J. CUNLIFFE

Summary.—The outermost part of the epidermis, including part of the pilo-sebaceous apparatus, has been sampled, using a cyanoacryalate adhesive, in patients with acne of varying severity, controls, and in acne patients treated with long term tetracycline. At the site sampled (left upper back) patients with acne have an increased amount of inspissated material as compared with controls; this appears increased in relation to the severity of the acne. The inspissated material, which is partly keratin and partly lipid, could possibly obstruct the outflow of sebum from the duct.

THERE is considerable evidence to suggest that obstruction to the outflow of sebum is important in the pathogenesis of acne. It is known that hyperkeratosis of the pilo-sebaceous duct is one of the earliest histological features of this condition (Van Scott and MacArdle, 1956); furthermore, certain fractions of sebaceous liquid, especially free fatty acids and squalene, when applied to rabbits' ears, promote follicular hyperkeratosis and comedone formation (Kligman, 1968; Kligman and Katz, 1968; Kanaar, 1971). On the other hand, the excretion rate of sebum of patients with untreated acne can be significantly increased by Sellotape stripping of the outermost layers of the stratum corneum (Cotterill *et al.*, 1972a). We sought to investigate possible factors affecting the outflow of sebum by using the stratum corneum sampling technique of Marks and Dawber (1971).

MATERIALS AND METHODS

A single drop of a polymer adhesive, ethyl cyanoacrylate (Permabond)* was placed on the unprepared skin of the back and immediately covered by a glass slide which was pressed in position for 20 seconds and then removed. The slide brings with it the adhesive, several layers of cells of the stratum corneum and attached hairs. Any remaining adhesive was then cleaned from the skin with acetone.

Samples from 130 individuals of either sex aged 15–30 were examined. Most samples were taken from the left side of the upper back; the face was avoided because of the serious complications should any of the cyanoacryalate adhesive enter the eye. Samples were taken from as apparently normal a skin as possible; most areas sampled contained only 1–2 lesions, and at worst the area sampled had no more than 3–4 follicles affected by a papule, comedo or pustule. The samples were taken between 10.00–12.30 hours. Table 1 shows the clinical source of the samples.

Control samples were from individuals with no sign of acne; the mid-lower back samples were included to represent an area of skin not usually subject to acne, and these samples were taken from both normal subjects and those with acne at other sites. All samples were examined using incident illumination at 12 × magnification with a

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* Obtainable from Staident Products Ltd., 33 Clarence Street, Staines, Middlesex, England.

TABLE I.—*The Clinical Source of the Samples*

Controls	30
Mild acne	40
Moderate and severe acne	18
Acne treated with long term tetracycline (poor response)	8
Acne treated with long term tetracycline (good response)	16
Mid lower back samples (normal appearance macroscopically)	18

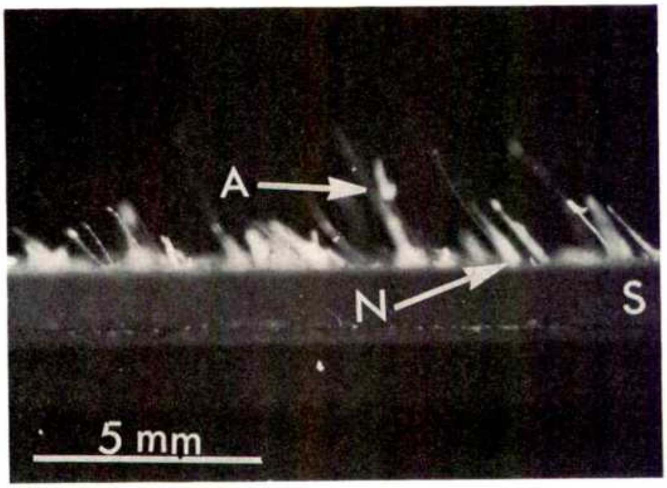

FIG. 1.—Permabond strip preparations on glass slide (S) viewed in the plane of the skin surface. Many of the hairs are out of focus, due to the extent of the strip. Apical or root material (A) can be easily distinguished from neck-shaft coating (N).

binocular microscope by the same individual (R.L.H.) without prior knowledge of their source.

The samples showed the pattern of skin creases modelled in the adhesive; the pilo-sebaceous ducts and hairs were visible. A proportion of the hairs were coated with opaque material (Fig. 1), referred to as " coating "; this occurred on the shaft or in the region of the neck of the follicle, and sometimes enveloped the whole hair.

The following data were assessed:

1. The total number of pilo-sebaceous units in 1 cm^2 of each sample.
2. The number of double, treble or multiple hair-containing follicles.
3. The number of coated units (irrespective of the number of hairs in the follicle).
4. The degree of coating; this was assessed subjectively, using a numerical scale ranging from 0 to 4.
5. The number of hairs measuring more than 1.5 mm in length in the area studied: they were pressed on to the surface of the slide and measured with an ocular grid.

No attempt was made to count the number of hairs in the various phases of growth, since, although almost all follicles contained one or more hairs, some of these were broken near the root, making it impossible to determine their phase.

The data obtained were grouped according to the origin of the sample. Attempts were also made to determine the nature of the coating material. For this purpose, whole strip preparations were stained with H. and E., or with Oil Red 0 or Sudan Black B to demonstrate lipids. Hairs with heavy coating were picked out individually, placed for about 40 min in toluol, and embedded in wax. Transverse sections through blocks of such specimens were cut and stained with either PAS-haematoxylin or Masson's trichrome. Similar samples were treated with buffered osmium tetroxide, embedded in Araldite, sectioned thinly, and examined by electron microscopy.

RESULTS

There was no significant difference in the number of hairs per cm^2 in the various samples, nor in the length of the hairs. There were, however, differences between the samples from the various groups in the percentage of coated hairs and in the degree of coating (Table II).

TABLE II.—*Percentage of Coated Units and the Degree of Coating (Mean±S.E.M.)*

	Coated units (%)	Degree of coating
(1) Controls	18·7±1·25	1·5±0·14
(2) MLB	20·1±2·8	1·1±0·08
(3) Mild acne	28·5±0·16	2·1±0·28
(4) Moderate and severe acne	35·1±4·3	2·9±0·72

Patients with severe acne showed a significantly greater number of coated units than those with mild acne ($p < 0{\cdot}01$), and samples from both groups showed a significantly greater number of coated units than samples from controls ($p < 0{\cdot}01$) and those from the mid lower back ($p < 0{\cdot}01$). Samples from patients with mild acne showed a greater degree of coating than samples from controls ($p < 0{\cdot}01$) and from the mid lower back ($p < 0{\cdot}025$). Samples from patients with severe acne showed an even greater degree of coating, as compared to those from controls and those from mid lower back ($p < 0{\cdot}025$).

TABLE III.—*Percentage of Coated Hairs and the Degree of Coating in Patients Treated with Antibiotics (Mean±S.E.M.)*

	Coated units (%)	Degree of coating
Poor response	27·5±5·4	2·5±0·49
Good response	21·5−3·0	1·8±1·00

Table III shows the number of coated units and degree of coating in patients treated with antibiotics. Although those who responded satisfactorily showed a reduced number of coated hairs and amount of coating, the differences were not significant.

In all the specimens examined a total of 450 follicles contained 2 or more hairs, while 3,800 contained single hairs. Sixty-one per cent of the units containing more than one hair were coated compared to 19% of the single hair units. There was, however, no significant difference in the proportion of multiple-hair-containing follicles in the various groups.

Nature of coating.—The sections of coated hairs examined by light microscopy showed that the coating in the neck region of the follicle contained a considerable amount of keratin and probably consisted largely of cells of the stratum corneum. The coating stained with both haematoxylin and with lipid stains (Fig. 2). The

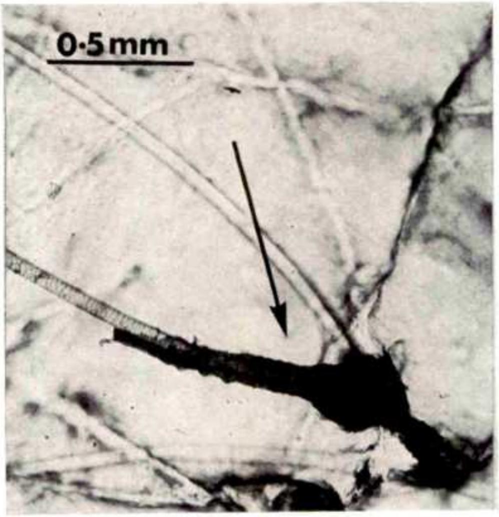

FIG. 2.—Coated hair of a strip preparation, stained by Sudan Black B and viewed by transmitted light. The neck and upper shaft of the hair are coated (↑), the deeper part of the hair uncoated. The coating material shows some positive staining for lipid.

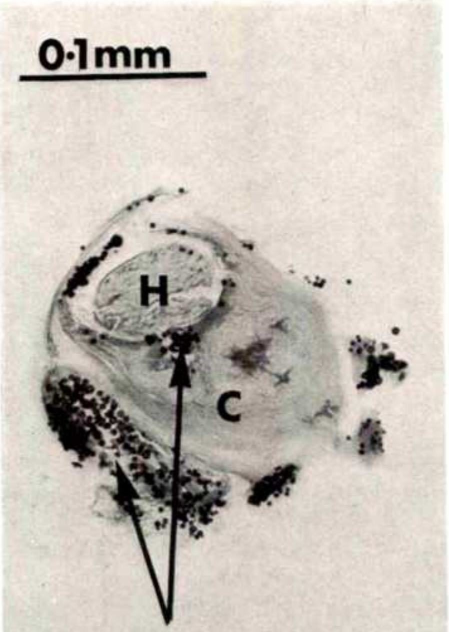

FIG. 3.—Low power electron micrograph showing laminae of keratin and amorphous material in the coating.

presence of keratin was confirmed by electron microscopy, which showed numerous layers of typical stratum corneum surrounding the hairs. There was also a considerable amount of amorphous material, as well as formed elements. These formed elements were of 2 types: the first resembled red blood cells in shape, but were generally smaller (*c.* 1·5–4·2 μm). They occurred in clumps, either between layers of corneum, or outside it (Fig. 3); they were PAS-positive and stained with haematoxylin. Much smaller round or ovoid granules were seen in the electron micrographs. These corresponded to bacteria in size and shape; in the histological preparations the only granules of this order of size detectable were those of melanin, which was observed within the hair shafts, or in some instances in the coating material.

DISCUSSION

Certain criticisms can be made of this useful technique of stratum corneum sampling; there were inevitably variations in the pressure applied to the glass slide and in the force necessary to remove the slide from the skin. These variations could possibly have modified the sample investigated; but, in view of the relatively clear-cut findings within each group, it seems unlikely that such variations are of much importance.

The observations indicate that patients with acne compared with controls have an increased proportion of coated pilo-sebaceous units, and that these show a greater amount of coating. These findings can also be related to the severity of the acne. Skin from the upper back of control subjects resembled that taken from the mid lower back, where acne does not usually occur. Although the precise composition of the coating is not yet clear, it does seem that keratin constitutes an appreciable part, together with lipid, possibly pigment (which agrees with the observations of Blair and Lewis, 1970), and other material not yet identified. Plewig *et al.* (1971) have demonstrated that a considerable part of the structure of the comedo, including microscopic comedones, arises from the follicular epithelium. Further work on this problem is in progress. The increased amount of coating in an area of skin which appeared comparatively normal in patients with severe acne elsewhere suggests that in many pilo-sebaceous units there is an accumulation of coating material which could act as an obstruction to the outflow of sebum. In keeping with this possiblity is the observation that sebum excretion rate can be increased by stripping the outermost part of the stratum corneum with Sellotape (Cotterill *et al.*, 1972a). Whether the keratin is primarily of epidermal origin or from the hair root sheath is still uncertain.

It is now well established that patients with acne have a significant seborrhoea, and that the greater the severity of the acne, the greater the seborrhoea (Strauss and Pochi, 1962; Cunliffe and Shuster, 1969; Cotterill *et al.*, 1971; Burton and Shuster, 1971). Therefore it is possible that our finding of a greater degree of coating with more severe acne is a function of the associated seborrhoea.

Certain fractions of sebum, including squalene and wax esters, can induce follicular hyperkeratinization and comedone formation in rabbits (Kligman, 1968; Kligman and Katz, 1968; Kanaar, 1971), and these fractions are increased (significantly only in females) in the surface lipid of patients with acne (Cotterill *et al.*, 1972b). Kligman (1968), Kligman and Katz (1968) and Kanaar (1971) have shown also that certain free fatty acids induce follicular hyperkeratinization and comedone formation. We therefore might have expected to find a reduced coating in patients treated satisfactorily with tetracycline, since such patients have reduced surface lipid free fatty acids (Freinkel *et al.*, 1965; Beveridge and Powell, 1969). However, although there was a reduction in the coating and number of coated units, this was not significant. Nevertheless, this lack of correlation could possibly be explained by the type of free fatty acids rather than the total amount of fatty acids in the surface lipid. It might be that some fatty acids mediate obstruction and others inflammation. Therefore it is possible that the coating observed could be a reflection of the biochemical composition of the sebum. These observations emphasize the importance of resistance to outflow of sebum in the pathogenesis of acne.

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AGE AND SEX VARIATION IN SKIN SURFACE LIPID COMPOSITION AND SEBUM EXCRETION RATE

J. A. COTTERILL, W. J. CUNLIFFE, B. WILLIAMSON AND L. BULUSU

Summary.—The sebum excretion rate and skin surface lipid composition were determined in 193 male and female control subjects aged between 4 and 60 years. The sebum excretion rate, squalene and wax ester content of surface lipid were maximal in males aged 26–40. The percentages of surface lipid wax esters were greater in males than females at all ages, but the prepubertal peak of wax esters in males may be due to an epidermal contribution from sterol esters. There was no significant age or sex variation in the sum of the percentages of triglycerides and free fatty acids, but the degree of hydrolysis varied considerably with age. The free fatty acid content was greatest in the first decade in both sexes, when the sebum excretion rate is at the lowest levels. Cholesterol content of surface lipid rose to maximal levels at puberty in both sexes.

It is important to identify factors controlling the biochemical composition of sebum and skin surface lipid since the composition of sebum may be important in the pathogenesis of acne (Strauss and Pochi, 1965). It seemed possible that useful information on such factors could be obtained by an investigation of a control population free of acne, but at present limited data only is available (Nikkari, 1965; Ramasastry *et al.*, 1970).

Forehead skin surface lipid composition was therefore determined in a large group of subjects free of acne. Forehead skin surface lipid was sampled by absorbent papers so that the rate of sebum excretion could be measured simultaneously.

SUBJECTS AND METHODS

One hundred and ninety-three subjects aged 4 to 60 years were investigated. These subjects had no evidence of clinical acne. A past history of acne, or acne scarring, clinically excluded subjects from further study. None of the subjects was receiving any systemic or topical therapy at the time of the investigations.

The following age groups were investigated:

1. Range 0–10 years: 17 males (age range 4–10 years, mean 7·7) and 8 females (age range 7–10 years, mean 8·6).
2. Range 11–15 years: 35 males and 49 females. Twenty-five of the female subjects had established menstrual bleeding and had a mean age of 13·9. The remaining 24 female subjects were awaiting the menarche and had a mean age of 12·4.
3. Range 16–25 years: 9 males and 27 females.
4. Range 26–40 years: 8 males and 11 females.
5. Range 41–60 years: 6 males and 23 females.

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The sebum excretion rate was determined from the forehead, using absorbent papers, by the method of Strauss and Pochi (1961) as modified by Cunliffe and Shuster (1969a). From the same samples the composition was determined semiquantitatively for squalene, wax and sterol esters, triglycerides, free fatty acids and cholesterol by thin-layer chromatography (Cotterill *et al.*, 1971a). All samples were analysed within 24 hours of collection to avoid any possible changes in the composition of the sample due to storage (Downing, 1970). Surface lipid was collected at the same time of day to avoid any changes produced by circadian variation, either in sebum excretion rate (Burton *et al.*, 1970) or skin surface lipid composition (Cotterill *et al.*, 1971b).

RESULTS

The results are shown in Table I and Fig. 1, 2.

Sebum Excretion Rate

Males aged between 0–10 and 11–15 have a significantly lower sebum excretion rate than males aged between 16 and 25 ($p < 0{\cdot}001$); 26–40 ($p < 0{\cdot}001$); and 41–60 ($p < 0{\cdot}001$, Fig. 1). The rate of sebum excretion is significantly lower in males aged 11–15 when compared to females aged 11–15 with established menstrual bleeding ($p < 0{\cdot}001$). These females also have a significantly higher sebum excretion rate than females aged 11–15 awaiting the menarche ($p < 0{\cdot}01$). After the age of 15 the sebum excretion rate was greater in males than females, and continued to increase in both sexes, reaching a maximum between the ages of 16 and 40. Thereafter, the rate declined in both sexes, particularly in females (Fig. 1).

Squalene

Although the squalene content varied little with age and sex, there were certain similarities between the shape of the graphs for squalene and sebum excretion rate (Fig. 1). In both sexes the lowest squalene content was in the 11–15 age group (prepubertal in females). This was followed by an increase, reaching maximum levels between the ages of 26–40 in males; the squalene content thereafter decreased in the 41–60 age group. The squalene content in the group of males aged 26–40 was significantly greater than in the group of males aged 11–15 ($p < 0{\cdot}025$). There were no sex differences in forehead surface lipid squalene content.

Wax and Sterol Esters

The percentages of surface lipid wax and sterol esters showed no significant variation with age, but maximal levels were achieved in the groups of males aged respectively 0–10 and 26–40. Wax esters in males were generally greater than those in females (Fig. 1), but these differences were not statistically significant.

Triglycerides and Free Fatty Acids

There was a clear reciprocal relationship between the percentage of free fatty acids and triglycerides (Fig. 2). The sum of the percentages of the triglycerides and free fatty acids was higher in females than males, but the differences were not statistically significant, and there was no significant variation with age. The degree of lipolysis to free fatty acids, however, varied considerably with age. The

TABLE I—*Variation in Sebum Excretion Rate and Surface Lipid Composition* ($\pm$*S.E.M.*) *in 193 Control Subjects*

Age group (years)	0–10		11–15			16–25		26–40		41–60	
	Male	Female	Male	Female	Female	Male	Female	Male	Female	Male	Female
Sebum	0·22	0·27	0·29	0·34	0·59	0·98	0·70	1·05	0·85	0·78	0·47
Excretion rate $\mu g/cm^{-2}/min.$	±0·03	±0·04	±0·03	±0·03	±0·07	±0·14	±0·07	±0·11	±0·09	±0·11	0·07
Squalene %	12·6	11·0	11·0	11·0	11·9	12·6	12·6	14·7	12·9	12·2	12·6
	±1·0	±1·8	±0·7	±0·6	±1·0	±2·5	±0·5	±1·3	±0·9	±0·6	±0·7
Wax and sterol esters %	29·9	24·1	26·2	24·5	25·5	27·3	24·9	30·0	25·0	27·9	25·6
	±2·1	±2·7	±1·0	±1·2	±1·3	±0·7	±0·9	±1·9	±1·8	±1·0	±1·1
Triglycerides %	32·1	36·3	47·5	49·6	48·1	41·6	47·7	44·5	44·4	38·9	43·9
	±3·9	±6·9	±2·1	±1·8	±2·8	±2·9	±2·3	±2·6	±2·8	±3·5	±3·0
Free fatty acids %	25·2	27·9	13·0	14·0	12·9	17·9	14·2	10·4	17·2	20·7	16·6
	±4·1	±9·7	±2·3	±1·9	±2·6	±3·6	±2·3	±2·7	±3·5	±3·7	±2·2
Cholesterol %	0·7	0·8	0·9	1·0	1·6	0·5	0·5	0·3	0·3	0·2	0·6
	±0·2	±0·2	±0·1	±0·1	±0·4	±0·1	±0·1	±0·1	±0·1	±0·1	±0·2

free fatty acid content fell and the triglyceride content rose in both sexes at puberty, and remained at these pubertal levels thereafter. The changes in surface lipid free fatty acids and triglycerides were statistically significant only in males, however.

Cholesterol

Peak values for cholesterol occurred in both sexes between the ages of 11 and 15 (Fig. 2). Males aged between 41 and 60 had a significantly lower percentage of surface lipid cholesterol than males aged 11–15 ($p < 0{\cdot}01$).

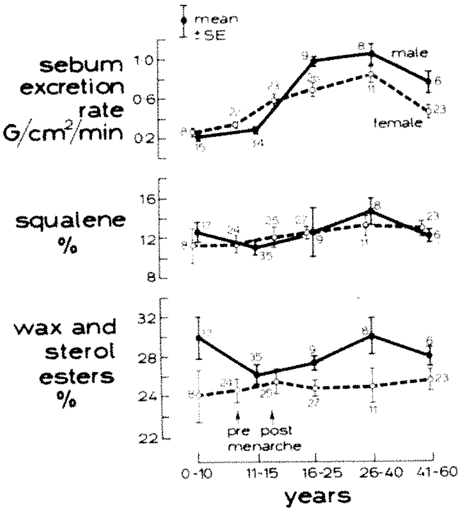

FIG. 1.—Age and sex variation in sebum excretion rate and in the percentages of surface lipid squalene and wax and sterol esters.

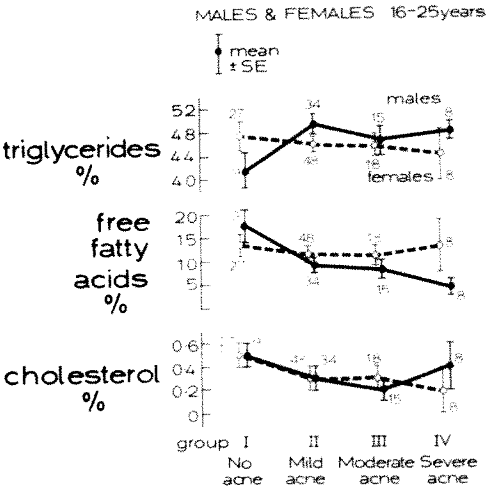

FIG. 2.—Age and sex variation in the percentages of surface lipid triglycerides, free fatty acids and cholesterol.

Females aged 11–15 with established menstrual bleeding had a significantly greater percentage of surface lipid cholesterol than females aged 16–25 ($p < 0{\cdot}001$); 26–40 ($p < 0{\cdot}005$); and 41–60 ($p < 0{\cdot}005$). The differences in the percentage of cholesterol in the 2 different groups of females aged 11–15 were not significant ($p > 0{\cdot}05$), but females with an established menstrual cycle had a significantly greater percentage of surface lipid cholesterol than males aged 11–15 ($p < 0{\cdot}05$).

Menstrual Cycle

There was no significant variation in either the sebum excretion rate or forehead skin surface lipid composition in relation to the menstrual cycle.

Correlation Between Sebum Excretion Rate and Forehead Skin Surface Lipid Composition

There was no significant correlation between the sebum excretion rate and the forehead surface lipid constituents studied in either sex, with the exception of a significant correlation between sebum excretion rate and surface lipid squalene content in females (correlation coefficient 0·20, $p < 0{\cdot}05$).

DISCUSSION

In this study the results for the sebum excretion rates showed a distribution similar to that reported by Cunliffe and Shuster (1969b). The finding by these workers of a greater rate of sebum excretion in females up to the age of 15 years is confirmed, and is likely to be due to the earlier onset of puberty in females. The significantly lesser sebum excretion rate in females awaiting the menarche compared to the post-menarche group is in keeping with this hypothesis, although the mean age of the latter group was 1·4 years greater than that of the former. After the age of 15, the sebum excretion rate is greater in males than females, and the rate rises to maximal levels in both sexes between the ages of 26 and 40, declining thereafter, but to a greater degree in females. Although the distribution of results was similar to that of Cunliffe and Shuster (1969b), the absolute values for the sebum excretion rates were higher in both sexes in this series. Both the age groups investigated and the technique used to determine the sebum excretion rate were similar to those of Cunliffe and Shuster (1969b) in Newcastle. It seems possible that regional variations in the rate of sebum excretion may occur, and this possibility merits further investigation.

Sebaceous gland activity in man is androgen-mediated (Strauss and Pochi, 1963), whilst oestrogens reduce sebaceous gland size, either by inhibiting the synthesis of endogenous androgen (Strauss *et al.*, 1962) or possibly by inhibiting the sebaceous gland (Ebling, 1963). There is, however, comparatively little data on the effect of these hormones on skin surface lipid composition in man. From studies in the rat it is known that surface lipid composition is determined by the secretory activity of the sebaceous glands, which in turn are stimulated by androgens, whereas oestrogens have no specific primary effect on surface lipid composition in this animal, although they may produce secondary changes by an effect on the secretory activity of the sebaceous gland (Nikkari and Valavaara, 1970). Pituitary hormones are also necessary for normal sebaceous gland function in the rat (Ebling *et al.*, 1969).

However, there are some difficulties in extending these studies to man because human skin surface lipid is rich in triglycerides, their hydrolysis products and squalene, all of which are present in negligible amounts in surface lipid of other animals (Nicolaides *et al.*, 1968).

In addition, the investigations which have been made in man are often difficult to interpret because of differing sites and methods of surface lipid collection. In this study the forehead was used as the sampling site because forehead surface lipid is mostly sebaceous in origin, with an epidermal contribution of less than 10% (Greene *et al.*, 1970).

A knowledge of the origin of the various surface lipid fractions may facilitate interpretation of changes in surface lipid composition. Squalene and wax esters are predominantly sebaceous in origin (Ramasastry *et al.*, 1970), whilst triglyceride is derived from both epidermis and sebaceous glands. Free fatty acids are derived from triglycerides by lipolysis, either by skin micro-organisms (in particular *Corynebacterium acnes*) (Freinkel and Shen, 1969) or by esterases present in the sebaceous ducts (Nicolaides and Wells, 1957). Cholesterol esters and cholesterol are predominantly epidermal in origin (Freinkel and Kazuo, 1969; Green *et al.*, 1970).

Studies in man have indicated that both the squalene content (Nicolaides and

Rothman, 1955) and wax ester content (Ramasastry *et al.*, 1970) of surface lipid increase at puberty and may therefore be androgen-dependent. In addition, it is known that testosterone will stimulate wax ester synthesis in the isolated mouse preputial gland (Sansone *et al.*, 1971). From a study of surface lipid composition of the female back during the menstrual cycle it appears that oestrogens, and possibly luteotrophic hormone, or both, influence the surface lipid cholesterol content, whilst the triglyceride content appears to be dependent on both oestrogens and progesterone (MacDonald and Clarke, 1970).

Surface lipid cholesterol is primarily epidermal in origin (Green *et al.*, 1970) and both androgens and oestrogens are known to stimulate epidermal mitosis (Bullough *et al.*, 1967). In the present study maximal levels of surface lipid cholesterol occurred in both sexes at puberty. Females with established menstrual bleeding had a greater percentage of surface lipid cholesterol than pre-menarche females, and significantly greater levels than males aged 11–15. These results support the hypothesis that oestrogens are important in cholesterol synthesis by the female skin, whilst the peak percentages of cholesterol in males around puberty may be androgen-dependent. The decrease in the cholesterol content of surface lipid after puberty may reflect increasing dilution of epidermal lipid by an increased volume of sebaceous lipid.

Can the variation in forehead skin surface lipid composition be explained, as in the rat, by changes in the secretory activity of the sebaceous glands? (Nikkari and Valavaara, 1970). The sebum excretion rate in man is an indirect measure of sebum excretion (Cunliffe and Shuster, 1969b). Because squalene and wax esters are predominantly sebaceous in origin (Ramasastry *et al.*, 1970) the content of these fractions in surface lipid may be expected to correlate with the rate of sebum excretion. Indeed, the percentage of surface lipid squalene is maximal in both sexes between the ages of 26 and 40, when the sebum excretion rate is also maximal.

The interpretation of the wax ester data is more difficult. The thin-layer chromatographic technique employed (Cotterill *et al.*, 1971a) does not satisfactorily resolve wax and sterol esters, and these lipid fractions are determined together. Because, in the normal adult, sterol esters are present in surface lipid in negligible amounts, and usually less than 3% (Downing *et al.*, 1969), it is usually not necessary to use specialized thin-layer chromatographic techniques to separate sterol and wax esters. However, it was shown after the inception of the present investigation that sterol esters, which are predominantly epidermal in origin (Freinkel and Kazuo, 1969), are present in appreciable percentages in prepubertal surface lipid and to a level of almost 15% in children aged 4–8 (Ramasastry *et al.*, 1970). In the present study the wax esters in males showed 2 peaks. The prepubertal peak is likely to be due to a significant sterol ester contribution, whilst the peak in the 26–40 years age group may reflect maximal sebaceous activity and a true increase in the synthesis of wax esters at this time. The lack of correlation between sebum excretion rate and wax ester content in females may be explained by changes in sterol esters masking real changes in surface lipid wax esters.

There was no significant age or sex variation in the sum of the percentages of surface lipid triglycerides and free fatty acids, which suggests that this fraction forms a similar proportion of both sebum and epidermal lipid. There was a clear reciprocal relationship between the percentages of free fatty acids and triglycerides, and this observation is in agreement with that of earlier workers (Nicolaides and Wells, 1957; Downing *et al.*, 1969) and supports the hypothesis that free fatty acids are derived exclusively by hydrolysis of triglycerides. There was, however, considerable variation with age in the degree of hydrolysis of triglycerides. Skin surface free fatty acid content is maximal in both sexes before the onset of puberty and falls to adult levels between the ages of 11 and 15 in both males and females. Corresponding reciprocal increases in the percentages of surface lipid triglycerides are also found at this time. The surface lipid changes, however, were statistically significant only in males because of wide individual variation in the degree of hydrolysis of triglycerides in prepubertal females. It is possible that the increased sebum excretion rate found in the older age groups studied may explain the changes in the degree of hydrolysis of triglycerides. An increased rate of sebum excretion may allow for less time for hydrolysis of triglycerides to free fatty acids by lipases present in the skin microflora or by sebaceous duct esterases. However, the decrease in the percentage of surface lipid free fatty acids in males aged 11–15 preceded any significant increase in sebum excretion rate towards adult levels. As changes in skin surface lipid composition towards the adult pattern are largely complete by the age of 9 years in American children (Ramasastry *et al.*, 1970), it could be that the degree of hydrolysis of triglycerides to free fatty acids is androgen-dependent and may be an earlier feature of puberty than an increase in sebum excretion rate.

Differing pathways of androgen metabolism within the skin may explain some of our findings. It is known that 17β-hydroxysteroid dehydrogenase (17β-HSD) activity is increased in the 20–30 age group and is found more frequently in male than in female skin biopsies (Calman *et al.*, 1970). Although the significance of this finding is not clear, it may be associated with the post-pubertal enlargement of sebaceous glands and their functional maturity at this age (Calman *et al.*, 1970). This observation may be relevant also to the present findings of age and sex variation in both sebum excretion rate and skin surface lipid composition.

While some of the age and sex changes in surface lipid composition may be explained in terms of variation in the sebum excretion rate, the alternative hypothesis that primary changes in sebaceous lipid composition may have a secondary effect on the sebum excretion rate must also be considered. It has been claimed that squalene, and to a lesser extent free fatty acids, influences the fluidity of sebum (Lewis and Hayward, 1971). Either an increase in squalene content or a decrease in the percentage of free fatty acids leads to an increase in lipid fluidity. The surface lipid squalene content was maximal in both sexes when the sebum excretion rate was also maximal. In addition, the free fatty acid content of surface lipid fell at puberty in females when the sebum excretion rate had increased significantly compared to prepubertal levels. Thus it is possible that primary changes in sebum composition may influence the sebum excretion rate. However, in males aged 11–15 the decrease in free fatty acid content compared to prepubertal levels was not associated with any significant increase in sebum excretion rate. Moreover, studies in patients with acne and subjects with a previous history of acne indicate that primary changes in surface lipid composition within the physiological range are unlikely to alter significantly the rate of sebum excretion in man (Cotterill *et al.*, 1972).

We conclude that marked age and sex variations in both sebum excretion rate and skin surface lipid composition occur and that these changes may reflect alteration, not only in endocrine function, but also in peripheral response to hormonal stimulation during life.

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LICHEN PLANUS; AN INBORN ERROR OF METABOLISM

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Summary.—The Km of the enzyme glucose-6-phosphate dehydrogenase for its substrate glucose-6-phosphate has been determined in normal human skin and skin from lichen planus patients. There was a clear difference between the 2 groups comparable to that found in congenital erythrocyte glucose-6-phosphate dehydrogenase deficiency. The results are discussed in relation to the aetiology and clinical picture of lichen planus.

THE aetiology of lichen planus has been the subject of speculation for a number of years. For a long time the infective aetiologies have received the most support, divided fairly evenly between bacterial and viral hypotheses. The infective theories have not been very satisfactory, since cross infection has never been reliably demonstrated, and the instances of conjugal lichen planus are less common than consanguinous ones. Also conjugal " familial " lichen planus occurs only at the rate to be expected from chance distribution in the general population (Hellier, 1950). Recent histological (Black and Wilson-Jones, 1972) and electron microscopic (Van der Staak, 1972) studies find no evidence of infection, but do locate the primary effect within the epidermis rather than the dermis.

Speaking of the lichenoid eruptions that occur as reactions to various drugs, heavy metals and photographic colour developers, Samman (1968) says: " A condition similar to lichen planus is caused by a number of drugs . . . and any theory of causation must take this into consideration ".

There is another well documented disorder that is precipitated by a similar range of compounds; the haemolytic anaemia of Favists, subjects with an erythrocytic glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) (G-6-PDH) deficiency. We have previously shown that the compounds known to produce haemolysis in these subjects are all inhibitors of G-6-PDH (Cotton and Sutorius, 1971), and we have suggested that, in subjects with an inherent deficiency in this enzyme, the added insult of these drugs will precipitate the haemolytic crisis. Thus we were led to the hypothesis that lichen planus might be a parallel disorder in which the G-6-PDH deficiency occurs in the skin, and that individuals with a subclinical deficiency could be precipitated into an eruption by antimalarial drugs, heavy metals, photographic colour developers or other inhibitors of G-6-PDH.

We have therefore examined the kinetics of this enzyme in the skin of lichen planus patients and normal control subjects; our findings are reported below.

MATERIALS AND METHODS

Skin biopsies.—Four mm punch biopsies were taken from the inner surface of the wrist (a site of predilection for lichen planus), from the buttock (an uncommon site for lichen

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planus) and from the upper arm of normal subjects and lichen planus patients. In the patients some biopsies were from lesions and some from clinically normal skin. The site to be biopsied was frozen with a " chlooraethyl " spray (Dr Lamers and Dr N. V. Indemans); the biopsy was taken with a stainless steel biopsy punch and placed on filter paper moistened with physiological saline in a sealed container at 0–4°C until used, generally within one hour.

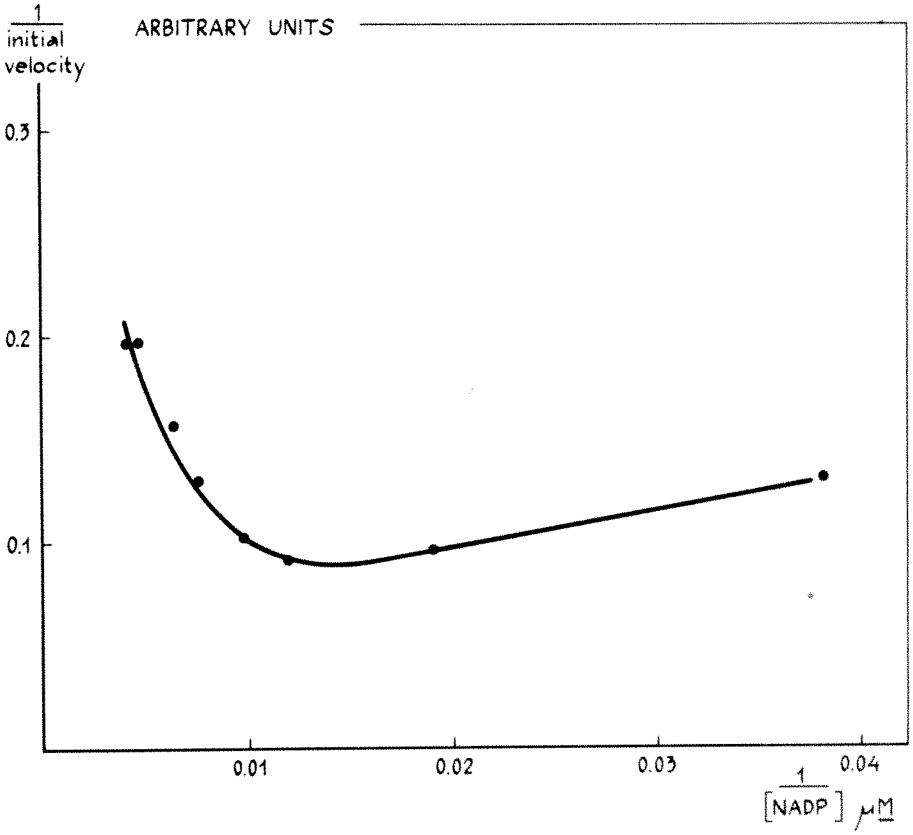

FIG. 1.—Lineweaver and Burk plot for glucose-6-phosphate dehydrogenase of normal human skin; at variable NADP concentrations.

The biopsies were weighed and homogenized in 2·5 ml tris 0·05 mol/l pH 7·3 buffer containing 5×10^{-4} mol/l EDTA + 3×10^{-3} mol/l $MgCl_2$. The homogenization was carried out in an all-glass microwet grinder (Townson and Mercer) fitted with an ice jacket. The assays were carried out under double blind conditions and the code was broken only at the end of the series.

Guinea-pig skin.—A series of duplicates of guinea-pig skin from different sites was assayed in the same way as the human material (in order to determine the reproducibility of the technique). Heavy metals, antimalarials and sulphonamides were tested as inhibitors, since the pure enzyme used in the previous study (Cotton and Sutorius, 1971) was a preparation from yeast.

Reagents.—The chemicals used were of analytical grade throughout and the NADP and G-6-P (glucose-6-phosphate) were the purest grade available from Sigma.

Assays method.—The skin homogenates were added to a 0·5 ml quartz cuvette containing a mixture of NADP, G-6-P and buffer, and were incubated at 37°C in a Baird Atomic spectrophotofluorimeter (excitation 340 nm and fluorescence 460 nm). One series of measurements was made for each homogenate, using different NADP concentrations in the range 10 μmol/l, to 260 μmol/l at a G-6-P concentration of 660 μmol/l and a second series using different G-6-P concentrations in the range 130 μmol/l to 660 μmol/l at an NADP concentration of 260 μmol/l. In each measurement the fluorescence due to the NADPH produced was recorded directly on a flat bed recorder (Servogar) for a period of about 5 min, and the initial velocities obtained by drawing tangents to these lines. The initial velocities were plotted as their reciprocals against the reciprocals of the NADP and G-6-P concentrations. These "Lineweaver and Burk" plots yielded apparent Km values for the enzyme in the crude homogenate.

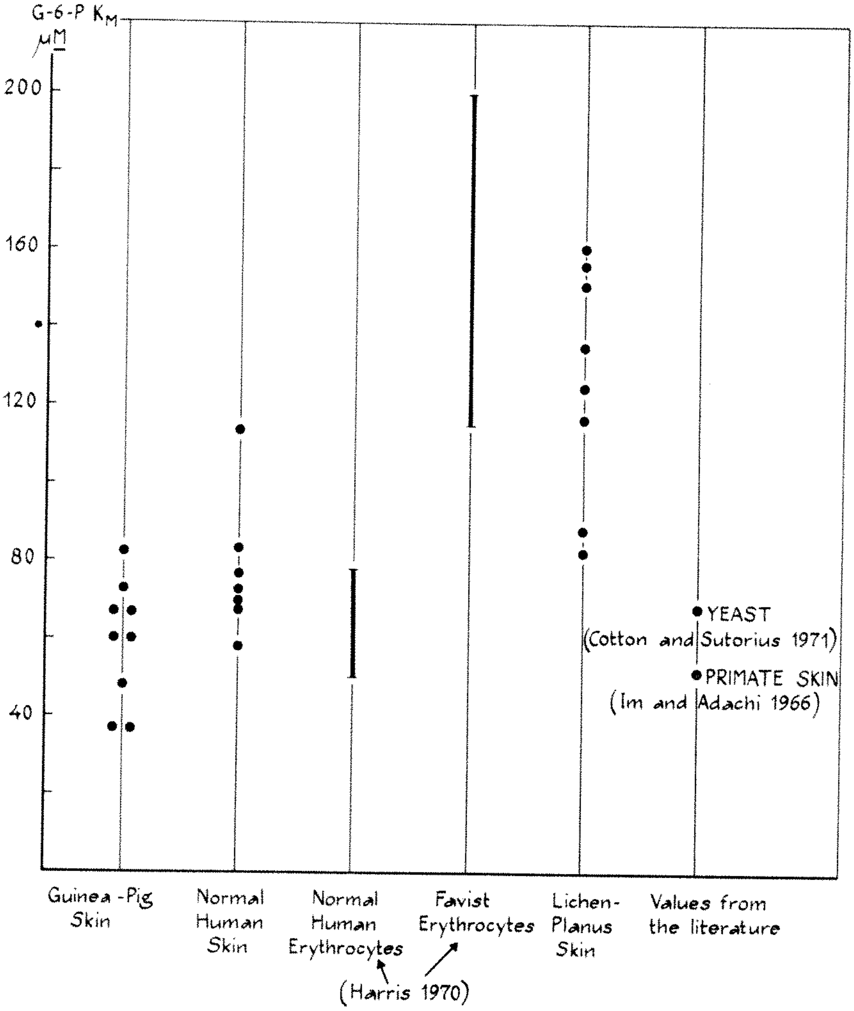

FIG. 2.—Apparent G-6-PDH Km values for G-6-P in normal human and lichen planus skin compared with guinea-pig skin and some values from the literature.

RESULTS

Guinea-pig skin.—The apparent Km values for G-6-P from different sites on various guinea-pigs are shown in Table I.

TABLE I.—*Kinetic Data from Guinea-pig Skin G-6-PDH from Different Sites*

Site	Vmax (μmol/g skin/min)	Km NADP μmol/l	Km G-6-P μmol/l
Scalp	0·295	38·5	66·7
Ear	0·733	35·2	60·2
Upper Forelimb	0·274	23·8	37·5
Lower Forelimb	0·198	25·4	71·4
Forepaw	0·188	34·0	82·0
Back	0·347	30·8	61·0
Upper Hindlimb	0·218	30·8	36·5
Lower Hindlimb	0·545	27·1	66·0
Hindpaw	0·601	34·0	48·8

Human skin.—The Lineweaver and Burke plots for NADP showed deviations from linearity which appeared to indicate substrate inhibition at high concentrations of NADP (Fig. 1). The degree of this inhibition was variable and not clearly associated with disease, site, sex or age, but prevented the accurate determination of Km values.

The apparent Km values for G-6-P are shown in Fig. 2, together with the guinea-pig values and the values for normal and Favist erythrocytes. The results of the inhibitor studies confirmed that cutaneous G-6-PDH is sensitive to the same range of compounds as the yeast enzyme.

DISCUSSION

The standard approach to enzyme deficiencies in erythrocytes is to measure the enzyme activity at maximal substrate concentrations and to express this as enzyme activity per unit quantity of red cells. This method is intrinsically unreliable with skin, since the ratio of epidermis to dermis is very variable, particularly in disease states, and enzyme activities/unit weight of skin are very misleading. The determination of apparent Km values is independent of the concentration of the enzyme.

The reproducibility of the apparent skin Km values for G-6-P in a genetically homogenous strain of guinea-pigs was good. The difference between the apparent Km values from human normal and lichen planus skin was much greater than the range found in the guinea-pigs.

Generally the Km and the Vmax appeared to be greater in the wrist than in the buttock skin of both normal and lichen planus subjects. However, the apparent Vmax values may be related to differences in thickness of skin; the relative significance of Km and Vmax determinations in skin disease has recently been discussed at length (Cotton and Mier, 1972). The apparent Km values for G-6-P in lichen planus skin were higher than in normal skin, regardless of age, sex, site and whether the biopsy was taken from a lesion or not.

The Km of G-6-PDH preparations from various sources for G-6-P as reported in the literature are compared in Fig. 2. It can be seen that our values for normal skin are similar. It can also be seen that the ratio: lichen planus skin to normal human skin is about the same as Favist erythrocyte to normal erythrocyte. Thus, our data support the idea that lichen planus is associated with a congenital abnormality in the G-6-PDH of the skin. Similar abnormalities in the skin of Favists have been reported (Davidson *et al.*, 1963), but there appear to be no publications on the incidence of lichen planus in these subjects.

Can this abnormality in G-6-PDH satisfactorily "explain" the clinical disease? The following observations are relevant to this question:

1. Favism is more common in the tropics, where it is thought to be associated with some resistance to malarial infection (Harris, 1970), and it is known that these individuals show similar abnormalities in the skin, as mentioned above (Davidson *et al.*, 1963).

2. Antimalarial prophylaxis precipitates an haemolytic crisis in Favists (Harris, 1970), and antimalarial prophylaxis during World War II produced a large number of "symptomatic lichen planus" eruptions (Samman, 1968).

3. The haemolysis in Favists is thought to be associated with a lack of reduced glutathione in the erythrocytes, since the glutathione reductase system is NADPH-dependent. In skin the glutathione reductase system is very active, and reduced glutathione is thought to be the endogenous inhibitor of melanin formation (Halprin and Ohkawara, 1967). Thus, in lichen planus we might expect necrosis of epidermal cells (by analogy with haemolysis) to be the primary lesion, and pigmentation to be one of the sequelae. Necrosis of epidermal cells is often the earliest change seen histologically in lichen planus, and pigmentation is a common side effect. It is of interest that heavily melanotic melanosomes can often be seen electron microscopically in the lesions (Van der Staak, 1972). Recently a reduction in G-6-PDH activity has also been observed histochemically (Black and Wilson-Jones, 1972).

4. Since lichen planus shows marked site predilection, we would expect to find site variations in the enzyme, which can in fact be clearly seen in Table I. Also, the cloning effect found with G-6-PDH variants (Davidson *et al.*, 1963) might well explain the curious hexagonal nature of lichen planus papules.

Thus there seems to be a good *prima facia* case for saying that the basic lesion in lichen planus is an abnormal G-6-PDH molecule with an increased Km for G-6-P.

There is obviously a great need to extend these results by examining a large number of lichen planus subjects and their relatives, but the disease is relatively rare in European populations and accounts for only about 1% of the dermatological intake. It is also essential to study G-6-PDH electrophoretically in these subjects. We are currently following these lines of research and will report the findings in due course. It may be that, with a larger group of subjects, our results will be seen to be fortuitous, but this seems unlikely. Also, of course, the results must be confirmed in other laboratories independently; but, from our preliminary results, we feel that the work would be well worth doing.

We should like to thank Prof. J. W. H. Mali for helpful discussions and encouragement, and Dr P. D. Mier for his help and advice. We should also like to thank the Audiovisual Department of the Radboud Hospital for preparing the illustrations, and Mrs J. G. M. Schuurs-Milder and Mrs K. F. M. Thurlings-Schlimme, who prepared the manuscript.

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THE CONTROL OF EPIDERMAL THICKNESS*

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PART 2

Phase 3: Carcinomata

The evidence shows that, throughout Phase 2, the average chalone content of the epidermis steadily falls so that both the rate of mitosis and the rate of post-mitotic ageing steadily increase (Fig. 7). In theory, therefore, the average chalone concentration in a group of epidermal cells could fall pathologically and permanently below the critical level at which post-mitotic ageing normally begins. Possible causes could be the failure of the cells to synthesize enough normal chalone, a too rapid chalone loss across an abnormal cell membrane, or a failure by the cells to respond to the chalone that is present. The result would be an explosively growing mass of permanently mitotic cells.

Basically, this is the situation in the Phase 3 type of epidermal growth (Fig. 7), and the result is a carcinoma in which the single, most important characteristic is that rate (mitosis) > rate (cell death). However, the observed rate of tumour growth is usually far slower than might be expected, so that, for instance, a human epidermal carcinoma may take months or even years to double its mass (Weinstein and Frost, 1970). Within such a tumour cell death is common; thus, in carcinomata of the mouse forestomach (the stratified epithelium of which is controlled by epidermal chalone; Frankfurt, 1967, 1971), some 17–43% of mitotic cells are destined to become post-mitotic, and so die, as against 50% in the normal steady state.

The first reason for the relatively slow growth of carcinomata is evidently that the conditions for growth are far from ideal. Thus, in the central region of a growing carcinoma, there is commonly a deteriorating blood supply (Bullough and Deol, 1971a); in malignant tumours there are commonly chromosomal and other cellular abnormalities which may distort various cellular activities and so impede the process of mitosis; and there is increasing evidence that the cell membranes of the tumour cells are commonly so abnormal as to provoke an immune reaction which then damages or destroys the cells (Klein, 1971).

Thus a growing tumour may be regarded as a group of cells in which the mitotic rate is inherently so high that it is able to grow in spite of the severe environmental limitations and inhibitions.

It is clearly important to determine the chalone relations of epidermal and other carcinomata, and already certain generalizations are beginning to emerge. Some 11 diverse tumours (including 3 epidermal carcinomata) in 6 species (including man) have so far been studied in relation to the chalones of their tissues of origin (Bullough and Deol, 1971a; Bullough, 1971). With no exceptions the tumours contained, and were evidently actively synthesizing, the chalones of their tissues of origin; and with no exceptions, when the concentration of the appropriate chalone was artificially raised, the mitotic activity of the tumour cells was inhibited. Evidence from 2 tumours, an epidermal carcinoma (Bullough and Laurence, 1968b) and a granulocytic leukaemia (Rytömaa and Kiviniemi, 1968b), has suggested that their basic abnormality lay in the cell membrane, which allowed an excessive rate of chalone loss; in both of these tumours the intra-cellular chalone concentration was estimated to be 10% or less of normal.

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* This article is published in two parts. Part 1 appeared in the September issue of this journal, p. 187.

If this is indeed a general weakness of tumours, one consequence should be that, as the tumour mass increases, the chalone concentration in the limited body space will rise. The first result of this would be a progressively depressed mitotic rate within the tissue of origin, and this has been described with 2 epidermal carcinomata (Bullough and Laurence, 1968b; Bullough and Deol, 1971b) and with a granulocytic leukaemia (Rytömaa and Kiviniemi, 1970). The second result should be that the growth of the tumour itself will be retarded. This is in fact first seen in the interior of the tumour mass (Fig. 8) where, as growth proceeds, the chalone concentration evidently rises to such a level that the cells become post-mitotic and die in the normal manner, with or without tissue function (Bullough and Deol, 1971a). Thus, in an epidermal keratinizing carcinoma, the tumour centre becomes a mass of keratin (Bullough and Deol, 1971b), while, in an epidermal non-keratinizing carcinoma, the central cells simply die (Bullough and Laurence, 1968b).

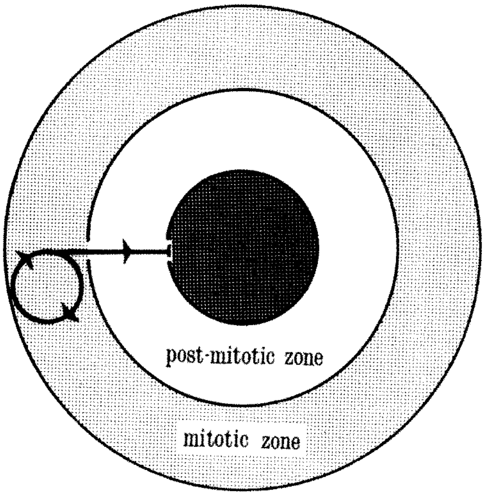

FIG. 8.—Diagram of a growing tumour in which the peripheral cells are mitotic and in which the older cells age normally to die centrally.

The final result of the rising chalone concentration should be the inhibition of growth of the whole tumour, and this may be a second reason for the slow growth of many tumours. Indeed, tumour growth commonly follows a sigmoid-type curve (Laird, 1964, 1969; Burns, 1969; Bichel, 1970, 1971). In a chronic tumour the growth actually ceases as the rate of cell death once more comes into balance with the rate of cell gain; in an acute tumour the animal dies before this balance has been achieved. In agreement with this theory, Laird (1964) has stated that

" tumours are still responsive . . . to some feedback control "; Burns (1969) has concluded that tumours " regulate their own growth as normal . . . tissues do so by the production of a homologous specific mitotic inhibitor "; and Bichel (1971) has produced important evidence for the existence of an autoregulatory mechanism of tumour growth " similar to the homeostatic growth regulation of some normal tissues."

The theory of the chalone control of tumour growth needs further confirmation by means of repeated chalone injections. So far, for technical reasons, this has been possible with only 3 tumours (2 melanomata, Mohr *et al.*, 1968; 1 granulocytic leukaemia, Rytömaa and Kiviniemi, 1969, 1970), but in all cases the tumour growth was either retarded or reversed. In many animals a complete cure was obtained.

Conclusions.—The characteristics of Phase 3 epidermal growth, derived partly from a study of epidermal carcinomata and partly from a comparison with the growth characteristics of other tumour types, are as follows:

(1) The rate of mitosis exceeds the rate of cell death and the result is a growing carcinoma which invades the dermis.

(2) There is evidence from one epidermal carcinoma (and a granulocytic leukaemia) that the high mitotic rate is due to the excessive rate of chalone loss from the cells. In 3 epidermal carcinomata (and in 8 other diverse tumours) an artificially raised chalone concentration inhibits mitotic activity, but each tumour reacts only to the chalone of its tissue of origin.

(3) As an epidermal carcinoma increases in size, there is an increasing concentration of epidermal chalone within the limited body space. This leads first to a progressive reduction in the general epidermal mitotic rate, and second to a reduction in the growth rate of the tumour itself.

(4) Commonly neither the mitotic rate nor the tumour growth rate is as high as might be expected. This is due partly to the rising chalone concentration within the body and partly to a variety of adverse physiological circumstances.

DISCUSSION

Other Chalone Mechanisms

It is clearly important to determine whether the epidermal growth control mechanism here described is also typical of other mitotic tissues. Certainly, tissue-specific anti-mitotic chalones have been found in every tissue in which they have been sought: sebaceous glands (Bullough and Laurence, 1970a), eccrine glands (Bullough and Deol, 1972), melanocytes (Bullough and Laurence, 1968c) granulocytes (Rytömaa and Kiviniemi, 1968a), erythrocytes (Kivilaakso and Rytömaa, 1971), lymphocytes (Lasalvia *et al.*, 1970; Garcia-Giralt *et al.*, 1970; Houck *et al.*, 1971), fibrocytes (Houck, 1971; and see Garcia-Giralt *et al.*, 1970), and in certain tissues of the liver (Scaife, 1970), kidney (Simnett and Chopra, 1969), lung (Simnett, *et al.*, 1969) and uterus (Hinderer, *et al.*, 1970).

Less is known of the actions of the various chalones on post-mitotic cell ageing, but it appears generally true that, the higher the mitotic rate, the quicker the post-mitotic cells die. Evidence regarding the sebaceous glands and the liver is given above (Tables I, II, II); evidence regarding the vaginal lining is given by Peckham and Kiekhofer (1962). Even in early embryos, local increases in the mitotic activity are accompanied by increases in the cell death rate (Glucksmann, 1951).

The nature of the close connection between the mitotic rate and the rate of post-mitotic cell ageing is unknown. They are both clearly related to the chalone concentration, they change in unison, and, at least in the case of the epidermis, different types of the same tissue have characteristically different rates of post-mitotic cell ageing. There is also evidence that such a characteristic rate of ageing can be altered. Thus, when one type of epidermis is transplanted to a different body region, it may come to resemble the epidermis of that region; and Billingham and Silver (1963) have suggested that epidermal characteristics are constantly dictated by some influence emanating from the underlying dermis. More precise evidence comes from sebaceous glands treated with oestrogens, which, without significantly changing the mitotic rate, cause a great reduction in gland mass (Table IX). This shows that the rate of post-mitotic ageing can be separately altered and that mitotic control and ageing control, though normally linked together, may be discrete mechanisms.

TABLE IX.—*Relation between Mitotic Rate and Sebaceous Gland Mass in Various Hormonal States in the Female Rat*

Condition of rats	Mitotic rate	Gland mass	N
†Normal untreated controls	6·67 ± 0·88	301·3 ± 23·5	9
Oestradiol-treated for 4 weeks	6·50 ± 0·76§	129·7 ± 14·2*	10
Hypophysectomized for 4½ weeks	28·50 ± 6·31*	359·6 ± 45·0§	10
‡Ovariectomized controls	14·6 ± 1·3	286·4 ± 16·8	10
Testosterone-treated for 3 weeks	26·6 ± 1·4*	408·3 ± 25·1*	10
Oestradiol-treated for 3 weeks	14·2 ± 1·6§	153·7 ± 18·7*	10

* Significantly different.
§ Not significantly different.
† From Ebling (1955).
‡ From Ebling (1957b).

Tissue Function

Tissue function may occur early or late in the life of the post-mitotic cells. It occurs early (in A1, Fig. 2) in the tissues of the kidney and liver, which can therefore revert to mitotic activity when necessary; it occurs late (in A2, Fig. 2) in erythrocytes and granulocytes, which therefore cannot revert to mitotic activity; and in the external body tissues, such as epidermis, sebaceous glands, and hair, it occurs only after the death of the cells.

When tissue function is performed by the A1 type of cell, chalone action is typically strong, mitotic activity is low, and the post-mitotic cells survive for a long time. When tissue function is dependent on the A2 type of cell, or on dead cells, the chalone action is typically weaker, mitotic activity is higher, and there is a continuing production of more quickly ageing post-mitotic cells.

It is important to note that, although post-mitotic ageing and tissue function are normally associated, they are separable processes. In tumours that have lost the capacity for tissue function, the ageing of the post-mitotic cells continues in an apparently normal manner (Bullough and Deol, 1971a). The ageing of post-mitotic cells is evidently an active process which is closely controlled, perhaps by "ageing genes" (Bullough, 1967).

Mitotic Activity and Tissue Mass

The next question is whether the 3 phases seen in the relationship between epidermal mitotic rate and epidermal mass are also seen in other mitotic tissues. Clearly Phases 1 and 2 occur in sebaceous glands (Tables I, II), Phase 1 in the liver (Table III), and Phase 3 in many different tumours (Bullough and Deol, 1971a). Although much more information is needed, these tissues and tumours are so diverse as to suggest that these phases are indeed typical of tissues in general.

This would imply that, however low the mitotic rate may fall in Phase 1, no tissue can ever shrink below a certain mass; and, however high the mitotic rate may rise within Phase 2, no tissue can ever grow beyond a certain mass. Hormone-dependent tissues also follow the same rules: after ovariectomy the uterus shrinks into Phase 1 and then becomes no smaller, while, after treatment with an oestrogen (which may specifically neutralize the tissue chalone; Bullough, 1967), it enters Phase 2 and grows to an extent that is limited by the faster rate of post-mitotic cell ageing.

The Cell Membrane

It has been commonly believed that, in epidermis, the chalone concentration has the form of a gradient, high distally and low basally, which is caused by the continuing loss of chalone into the blood. This may be partly true, but it is equally possible that the gradient is related to a decreasing cell membrane permeability in the ageing post-mitotic cells. As an epidermal cell moves distally, the nature of the cell membrane changes radically (Vaughan and Bernstein, 1971); membrane permeability increases dramatically when a distal epidermal cell reverts to mitosis after damage (Bullough, 1966, 1969); the removal of the outer cover layer of the cell membrane by means of proteolytic enzymes causes a temporary loss of mitotic control (Burger, 1970); the modification of the cell membrane may be the "initial event" of the pro-mitotic action of phytohaemagglutinin on lymphocytes (Allan *et al.*, 1971); while, conversely, a reduction in cell membrane permeability may account for the widespread anti-mitotic action of glucocorticoid hormones (Bullough and Laurence, 1968a).

The state of the cell membrane may also be of basic importance in cancer. Rubin (1966) has suggested that "malignant transformation arises from a hereditable disruption in the distribution and configuration of cell surface molecules", and Markert and Ursprung (1971) have described "neoplasia as a disease of cell membranes". It is certain that tumour cell membranes may be so altered as to induce an immune reaction (Klein, 1971); that virus-induced cell transformation may be mediated by cell surface changes (Dulbecco, 1969); that in some tumours the altered cell membranes are incapable of retaining the chalone that is synthesized (Bullough and Laurence, 1968b; Rytömaa and Kiviniemi, 1968b); and that, when the appropriate chalone is forced back into the tumour cells, their mitotic activity is inhibited (Bullough and Deol, 1971a, b; Bichel, 1970, 1971).

The Chalone Mechanism and Cancer

Thus, the question arises as to whether the present conclusions concerning Phase 2 and Phase 3 epidermal tumours are relevant to cancer in general. The Phase 3 tumours so far studied in relation to their tissue chalones are: 3 epidermal carcinomata (in rabbit, Bullough and Laurence, 1968b; in guinea-pig, Elgjo and Hennings, 1971; Laurence and Elgjo, 1971; in mouse, Bullough and Deol, 1971b, 2 melanomata (in mouse and hamster, Bullough and 1968c; Mohr *et al.*, 1968), 2 granulocytic leukaemias (in rat and man, Rytömaa and Kiviniemi, 1969, 1970), 2 lymphocytic leukaemias (in mouse and man, Bullough and Laurence, 1970b; Houck *et al.*, 1971), a cervical carcinoma (in man, Hinderer *et al.*, 1970), and a plasmocytoma (in mouse, Bichel, 1970, 1971). All these tumours have proved similar: they all contain a tumour-specific antimitotic agent with the characteristics of the chalone of their tissue of origin, they all act as if their intracellular chalone concentration is too low, and they all respond by mitotic inhibition when treated with adequate amounts of the appropriate tissue chalone.

Indeed, the general conclusion now emerging is that, when treated with the chalone of its tissue of origin, a carcinoma or a leukaemia will stop growing; and that, if the treatment is properly planned, the tumour will shrink and may even disappear permanently. Probably the action of chalones on tumours, like that of antibiotics on bacterial infections, is to inhibit further cell division; the subsequent disappearance of the tumour, like that of an arrested infection, is evidently due to some other factor, such as an immune reaction. However, before this and other important problems can be adequately studied, techniques must be devised for the large scale production of pure enough chalone preparations (Bullough, 1971).

This analysis given here began in a series of stimulating discussions with Professor A. Tosti (Istituto di Dermatologia Sperimentale dell'Universita di Palermo). It is also a great pleasure to acknowledge the help of Dr E. Christophers (Dermatologische Klinik der Universität München), who provided the material for Figs. 4 and 5 and for Table VI.

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ULTRASTRUCTURAL STUDY OF THE CUTANEOUS ELASTIC FIBRES IN LUPUS ERYTHEMATOSUS

D. SCHMITT, J. THIVOLET AND H. PERROT

Summary.—During the ultrastructural study of the cutaneous lesions of 5 cases of systemic lupus erythematosus (S.L.E.) and 2 cases of discoid lupus erythematosus (D.L.E.), the authors report the changes observed in dermal elastic fibres.

These changes consist in a progressive disappearance of the granular matrix and in the subsequent appearance of the fibrillar component of the elastic fibres. These modifications of which several types are described, end in the formation of fibrillar bodies. The evolution of these changes is compared to the evolution of actinic elastosis.

Although histopathological abnormalities in the cutaneous lesions of lupus erythematosus are now well established, ultrastructural findings are few and fragmentary, with the exception of numerous studies of the virus-like inclusions.

Grishman and Churg (1970) and Tuffanelli *et al.* (1969) have described the changes in the dermo-epidermal junction and in the dermis. We have confirmed most of these ultrastructural abnormalities (Schmitt *et al.*, 1970). In S.L.E. we observe: necrosis of the basal layer of the epidermis, a very distorted basement membrane " en labyrinthe ", necrosis of dermal cells, modifications of the vascular walls, and some sparse fibrin deposits. In discoid lupus erythematosus, the changes in the dermo-epidermal junction are more pronounced. The polymorphonuclear leukocyte infiltration is more important, and the fibrin deposits are more frequent; but we have not observed the organized electron-dense deposits at the dermo-epidermal junction and in vessel walls as described by Grishman and Churg (1970).

Changes in electric fibres seem to have been suspected by Grishman and Churg, who describe " dermal fibrillar bodies ". In several cases of S.L.E. and D.L.E. we have been able to observe the elastic fibres in abnormal skin and in a few cases we have compared them to the elastic fibres of normal sun-exposed and non-exposed skin. In this way we have been led to observe a very distinctive pattern of changes in elastic fibres.

MATERIALS AND METHODS

(a) The materials were obtained by biopsy of specific cutaneous lesions—from 5 patients showing typical S.L.E. clinically, biologically and histoimmunologically (L.E. cells, antinuclear antibodies, hypergammaglobulinaemia, immunoglobulins and complement fixed at the dermo-epidermal junction); and from 2 patients suffering from

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D.L.E. In one case we have compared samples of normal sun-exposed skin (hand), normal covered skin (shoulder) and abnormal skin (face).

(b) The skin specimens were fixed immediately with glutaraldehyde 3% in cacodylate buffer 0·4 mol/l, pH 7·2 for 2 hours at 4°C, washed and post-fixed by osmium tetroxide 1%, with cacodylate buffer for one hour at 4°C. The specimens were embedded in Epon 812.

Sections 0·5 μm and 1·0 μm thickness were placed on slides and stained according to Richardson *et al.* (1960) with methylene blue and Azur II and examined by light microscopy. Fine sections cut with a Reichert ultra-microtome were placed on grids and contrasted according to Reynolds (1963).

The ultrastructural examination was performed with Hitachi HU-II-A, Hitachi HU-II-C, and Philips EM 300 microscopes.

RESULTS

With light microscopy, after staining with orcein the elastic fibres in abnormal skin appear irregular and fragmented. With electron microscopy, the abnormalities can be more precisely observed.

1—*Abnormal Skin*

The elastic fibres are always more or less modified. The abnormalities are common to the 2 types of lupus erythematosus, but the frequency of the different appearances varies from case to case. We classify the changes in 3 types, but all the intermediary forms are observed.

Type A: Spongy fibres.—This is the most frequent type. The elastic fibres have a heterogenous, granular matrix, composed of a pale ground against which a large number of electron-dense spots stand out. The outline of this matrix is irregular, finely indented and sometimes fragmented. At the periphery of the fibres some fibrils form an intricate halo which appears while the central dense matrix progressively disappears.

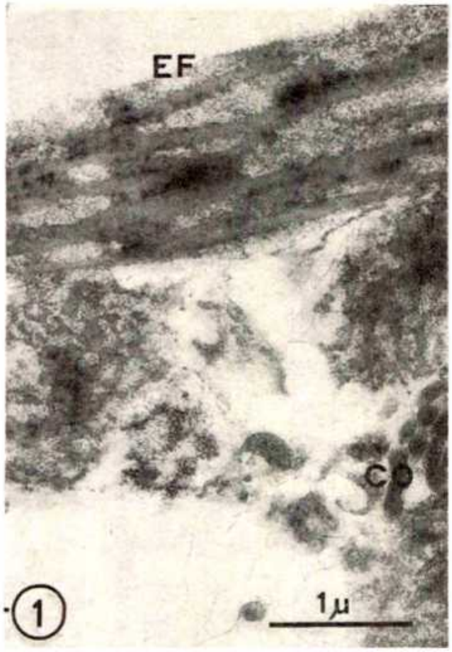
Type B: Intermediate stage.—This is more distinctly individualized. The dense central matrix is very much reduced, with a few dense spots and a finely indented outline. The fibrillar halo is very conspicuous.

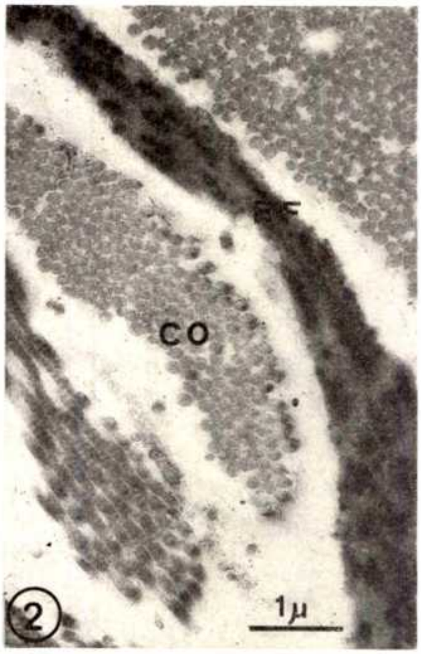
Type C: Fibrillar bodies.—In this type there is only one component left. In the dermis the fibrillar part forms irregular masses of various sizes, which are sometimes numerous. The fibrillar bodies are more frequent in D.L.E. Collagen is more frequent. The fibroblasts, which are rare, have frequently a pyknotic nucleus and an altered endoplasmic reticulum.

2—*Normal Skin*

In normal covered skin (shoulder) the elastic fibres are normal. They present 2 components: a finely granular dense matrix, with electron-dense spots, and a fibrillar component formed of fine fibrils, particularly visible on the periphery of the fibres.

In normal sun-exposed skin the elastic fibres are more dense than in non-exposed skin. The matrix is heterogenous: on a finely granular and pale ground there are some voluminous electron-dense spots, which give a spotted and striped appearance. The peripheral fibrils are more visible. The general aspect is that of an early actinic elastosis.





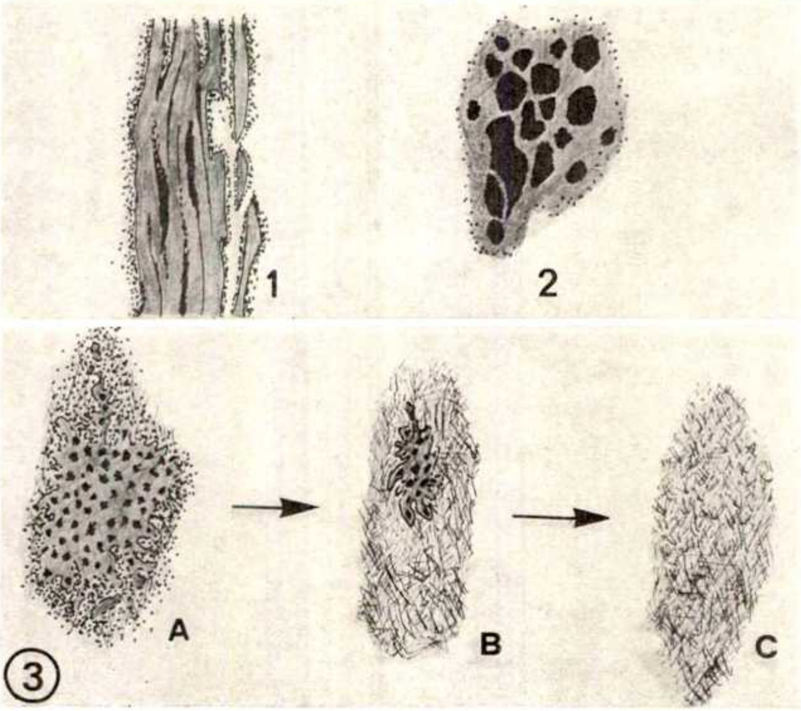
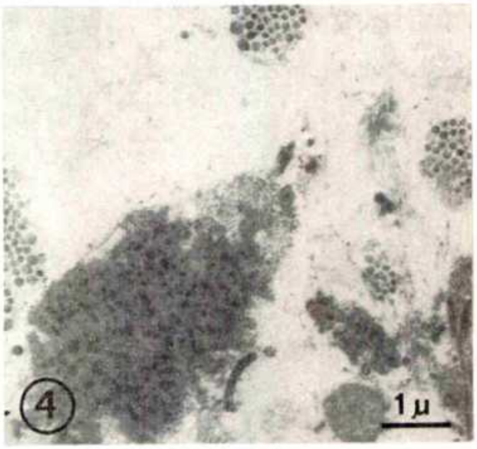
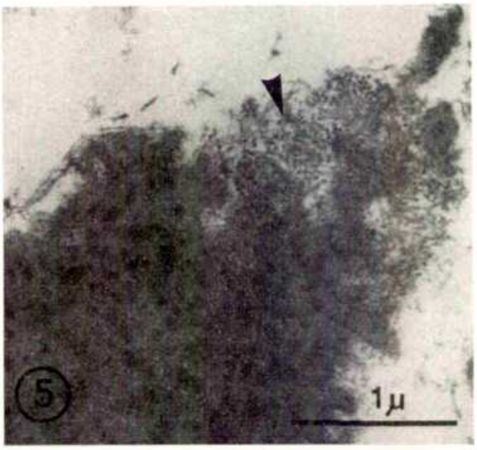


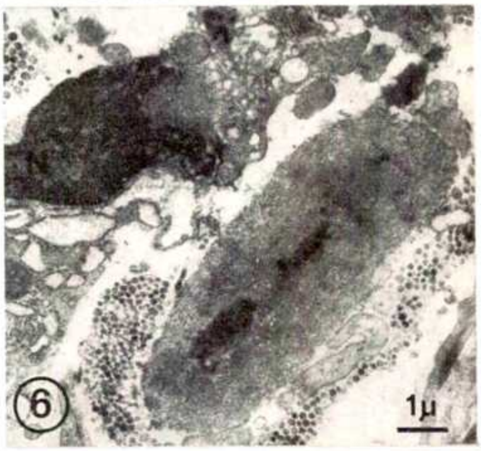
Fig. 1.—Appearance of the elastic fibres in normal non-exposed skin EF: Elastic fibre; CO: Collagen. (× 21,000.)

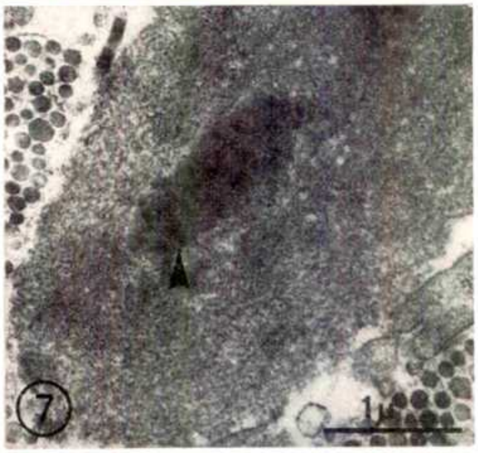
Fig. 2.—Apperance of the elastic fibres in normal exposed skin EF: Elastic fibres; CO: Collagen. (× 14,000.)

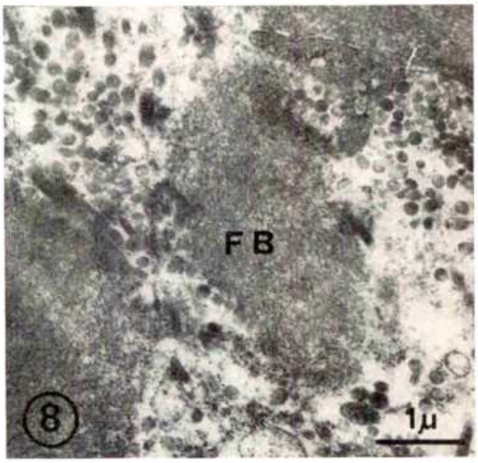
Fig. 3.—Elastic fibre changes in abnormal skin (lupus erythematosus) (Types A, B, and C) and apperance in normal non-exposed (1) and exposed skin (2).











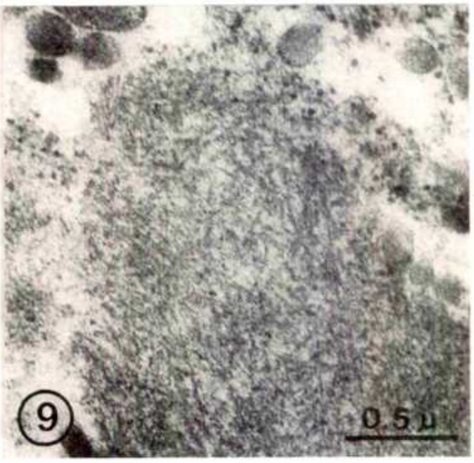


FIG 4.—Abnormal skin, Type A. Spongy appearance. (× 10,000.)

FIG. 5.—Detail of the previous figure, showing (arrow) the fibrillar support. (× 25,000.)

FIG. 6.—Abnormal skin, Type B. Note the necrosed dermal cell. (× 7,450.)

FIG. 7.—Detail of the elastic fibre of the previous figure, showing (arrow) the residuum of the central dense matrix. (× 20,000.)

FIG. 8.—Abnormal skin, Type C. Fibrillar bodies. (× 13,000.)

FIG. 9.—Detail of the previous figure, showing the delicate fibrillar support. (× 27,000.)

DISCUSSION

The different abnormalities of the elastic fibres (types A, B and C) which have been observed in the 2 forms of lupus erythematosus are represented on the same biopsy; their aspects are then polymorphic. The comparison between sun-exposed skin, either normal or abnormal, is easy because it can be carried out in the same area, while the comparison between normal covered skin and normal or abnormal sun-exposed skin requires the study of 2 different areas; the biopsy site must therefore be taken into account.

The ultrastructural aspect of elastic fibres, although it depends on the biopsy area, the species, the age and the experimental methods, is relatively well defined (Ross and Bornstein, 1969; Hashimoto and Di Bella, 1967). There are 2 components: a granular matrix and a fibrillar component which is visible on the periphery of the fibres. Desmosine and isodesmosine, which from the biochemical source of the fibres (Partridge *et al.*, 1965) are part of the dense matrix which biochemically corresponds to the elastin. The fibrillar component, that is to say the " structural glycoproteins " (Robert *et al.*, 1970), provides important support. According to these authors, it disappears progressively with the age of the individual, and it could even disappear totally. In the process of elastogenesis this fibrillar support also plays a part in the orientation and the polymerization of the fibre (Robert *et al.*, 1971). This elastogenesis process, which has been postulated by Farhenbach *et al.* (1966), has been confirmed by Jones and Barson (1971). In a preliminary stage some fibrillar masses appear in the vicinity of the connective tissue cells; in a second stage a dense granular matrix is deposited on these fibrillar masses. This central matrix progressively infiltrates the fibrillar support, which thus progressively disappears with ageing.

In lupus erythematosus in abnormal skin we observe the opposite process. It seems as if the component of the matrix progressively disappears (types A and B) not allowing anything but the glycoprotein fibrillar support which forms the fibrillar bodies (type C), to appear. The latter are described by Grishman and Churg (1970) and are interpreted as a non-specific degeneration of the dermis. They have been noted by Jarvi *et al.* (1969) in an elastofibroma.

The process of change in these elastic fibres consists in isolating the structural glycoprotein support at the expense of the disappearance of the biochemists' elastin. The pathological process may contribute to the understanding of the change in the elastic fibre and of its normal structure. The results of ultrastructural contrasting of the matrix components of the fibres, according to the technique employed by Jones and Barson (1971) on sections of biopsy embedded in Araldite, have remained negative.

The appearance of the elastic fibres in normal sun-exposed skin is the first stage of a physiological elastic degeneration process, which is that of actinic elastosis. In this case the component of the matrix is hypertrophied and dense. It ends in the formation of voluminous matrix masses, while the fibrillar component disappears progressively and can even totally disappear (Braun-Falco, 1965).

In the cutaneous lesions of lupus erythematosus we can see that the changes in the elastic fibres are approximately the opposite of those in actinic elastosis: the matrix hypertrophies at the expense of the fibrillar components, while in lupus erythematosus the matrix disappears and allows the fibrillar component to appear.

Finally, the study of the elastic fibres of the dermis in lupus erythematosus reveals some relatively distinctive alterations, which differ particularly from actinic elastosis. A further study ought to be undertaken to show whether or not these changes are specific to lupus erythematosus, and, above all whether a precise physio-pathological mechanism can be attributed to them.

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HAIR DIAMETER IN FEMALE BALDNESS

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Summary.—Twenty scalp hairs were plucked from each of 131 Caucasoid women, and measurements of diameter, at 1 mm intervals, starting just above the root, were made for each hair. There was little variation in diameter along normal anagen hairs in any of the subjects. The mean anagen hair diameter in 58 women with diffuse hair thinning, of whom 44 were without detectable endocrine abnormality and 14 were clinically hypothyroid, significantly decreased with age. This appeared to be an accentuation of a normal trend, although this could not be statistically established in the control group of 73 women. At any age the mean thickness of hair in the subjects with diffuse thinning was less than in normal subjects of the same age. Normal subjects showed a symmetrical distribution of hair diameter, with a marked peak at 0·08 mm. In contrast, subjects with diffuse alopecia without detectable endocrine abnormality showed a wide spread of diameters, with 2 equal peaks at 0·04 mm and 0·06 mm. The hypothyroid group showed a similar distribution, except that there was a larger proportion of hairs of the smaller diameters. The results suggest that, in the development of diffuse thinning, not all follicles behave identically, and that there may be more than one type of follicle in the human scalp.

SEVERAL types of baldness can be recognized in women. The present study is limited to subjects with a gradual and diffuse thinning over the vertex. Cases of alopecia areata, and post-partum or post febrile hair thinning, which is usually transient and caused by a sudden shedding of club hairs, or telogen effluvium (Guy and Edmundson, 1960; Kligman, 1961), have therefore been excluded.

Hair loss sufficiently severe to cause obvious thinning occurs in 2 main patterns. The first shows frontal recession, with some central thinning, and appears to have an hereditary component (Smith and Wells, 1964). The second shows diffuse thinning over the vertex, with retention of the normal hair line. A third pattern, in which such diffuse thinning is confined to an orbicular zone around the crown—" skull-cap alopecia "—also has been recognized by Ludwig (1964).

The causation of generalized diffuse thinning of the vertex, in contrast to hereditary pattern baldness, is still a controversial subject. Previous work (Eckert *et al.*, 1967) has shown that usually no endocrine abnormality can be demonstrated, although some cases are associated with hypothyroidism. Other suggested causative factors of diffuse thinning of hair include iron deficiency (Hård, 1963), the ingestion of amphetamines for weight reduction (Alexander, 1965), traction and trauma on the hair (Slepyan, 1958; Lipnik, 1961) and localized scalp disease such as seborrhoeic dermatitis. Ludwig (1964), however, has

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claimed that diffuse alopecia in women is indistinguishable in causation from common pattern baldness in men. Of 102 patients with diffuse alopecia, he found that 82 were daughters of bald fathers or of mothers with thin hair, or belonged to families in which male pattern baldness was a common trait.

Male pattern alopecia is characterized by a gradual regression of the hair to lanugo, and measurements of hair diameter show a gradual decrease (Barman *et al.*, 1969). In the present study hair diameter has been measured in women who have within recent years attended the Dermatology Department of the Hallamshire Hospital, Sheffield, with the complaint of thinning hair. Particular attention has been paid to possible differences between hypothyroid cases and those with no demonstrable endocrine abnormality.

PATIENTS AND METHODS

Fifty-eight Caucasoid women aged 13–72 with diffuse thinning were examined; 44 of these had no detectable endocrine abnormality, whilst 14 had previously been found to be clinically hypothyroid, as confirmed by P.B.I. estimations and radio-iodine tracer studies. A control group of 73 Caucasoid women, all with normal " heads of hair ", was also examined. Twenty hairs were plucked from the vertex of each individual with blunt-ended forceps at a point 10 cm posterior to the frontal hair line. The hairs were stuck on to Scotch tape which was then attached to a microscope slide. The number of anagen, telogen and dystrophic anagen hair roots (Van Scott *et al.*, 1957; Van Scott and Reinertson, 1957) were then counted. For each hair, 20 measurements of diameter, using a calibrated micrometer eyepiece, were made at 1 mm intervals, the first being just above the hair root.

RESULTS

In analysing the results, 3 questions need to be considered in relation both to the ageing of normal subjects and to the development of diffuse alopecia. Firstly, does each hair shaft have a constant diameter throughout its length and, if so, is the condition maintained? Secondly, does the average diameter become less, as in male pattern baldness? Thirdly, if so, is this true for all or only for some of the hairs?

Apart from a tapering at the tip, there appeared to be little variation in diameter along normal anagen hairs in any of the subjects. The " dystrophic " anagen hairs, present in very small numbers in all groups, also showed constant diameters, but often of a completely different order. Hairs in telogen, on the other hand, often showed a gradual decrease in diameter towards the root. For statistical analysis it was therefore decided to exclude telogen and the small number of dystrophic hairs, and to use only normal anagen hairs. From the average for each hair, the mean diameter was calculated for each subject.

Mean hair diameters are shown in Fig. 1 and the regression coefficients in Table I. The coefficient for the diffuse thinning without endocrine abnormality group ($n = 40$) is significantly different from zero ($t = 2{\cdot}69$; $p = 0{\cdot}01$ approx.); that for the hypothyroid group ($n = 14$) just fails to be significant at the 0·05 probability level ($t = 2{\cdot}15$); and that for the much larger normal group ($n = 71$) fails to reach significance ($t = 1{\cdot}83$). Thus, in the alopecia groups there appears to be a significant decrease in mean diameter with age, although this tendency is not statistically established for normal subjects. At the same time, none of the regression coefficients is significantly different from that obtained by fitting parallel regression

TABLE I.—*Regression of Mean Hair Diameter (mm) on Age (years)*

	n	Regression coefficient	S.E. of b.
Controls	71	−0·00017	0·000093
Diffuse thinning without endocrine abnormality	40	−0·00028	0·000108
Diffuse thinning associated with hypothyroidism	14	−0·00043	0·000200

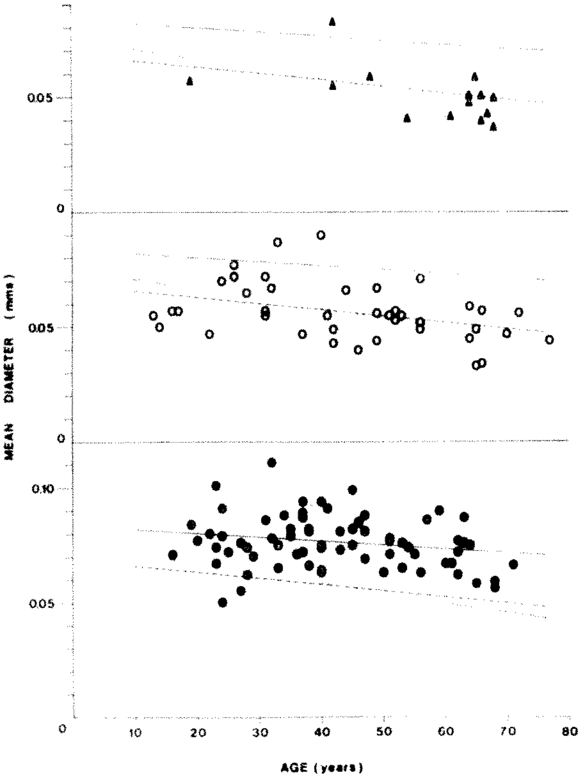

FIG. 1.—Mean hair diameter related to age———●———, normal subjects; – – – –○– – – –, diffuse thinning without endocrine abnormality; . . . . . .▲. . . . . ., diffuse thinning and hypothyroidism. The 3 regression lines are shown on each diagram.

to each of the 3 groups. The result can be interpreted to mean that hair diameter lessens with ageing in alopecia subjects and that this could be an accentuation of a normal trend, although this was not statistically established.

On the assumption that the regressions are parallel, it is possible to compare the group means corrected for this regression. Such an analysis reveals that the means for the diffuse thinning groups were each significantly less than the mean for the control group, but were not significantly different from each other.

Is the lowered mean diameter in subjects with diffuse alopecia due to thinning of all or only of some of the hairs? The range of diameters for hairs from 2 normal subjects is shown in Fig. 2. In each, almost 60% of the hairs were about 0·06 mm or 0·08 mm thick, and this picture was typical. Selected subjects with diffuse thinning without endocrine abnormality retained some hairs of this thickness, but most were much thinner; hence there was a far wider range of diameters (Fig. 2). By contrast, in 2 selected cases of alopecia associated with hypothyroidism, 80% of the hairs were approximately 0·04 mm thick, and there were virtually no hairs of the normal average.

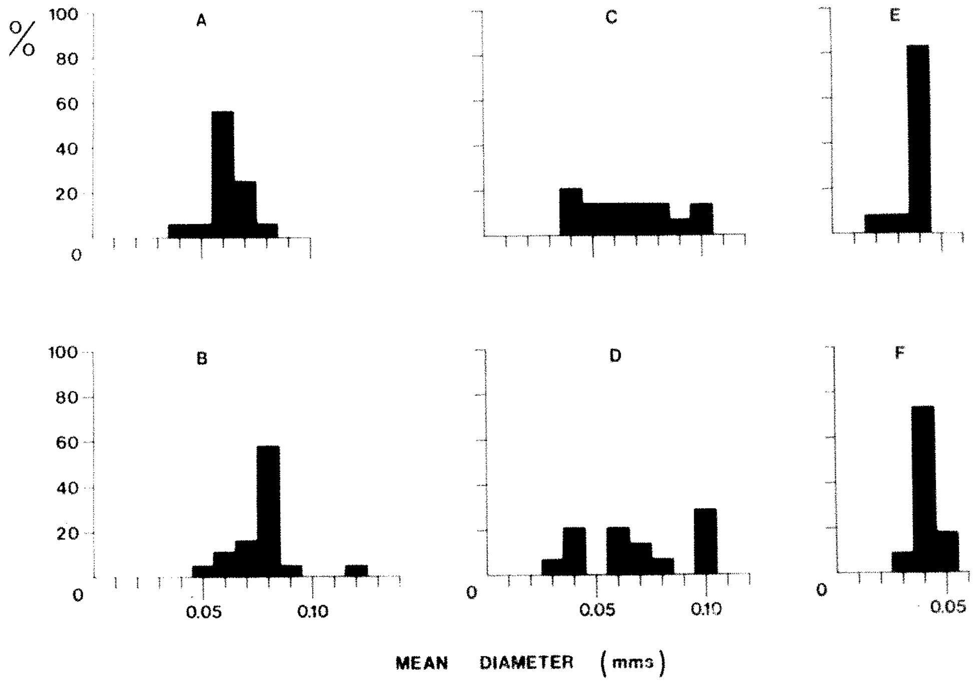

FIG. 2.—Variation in hair diameter in 6 selected cases. A and B, normal subjects; C and D, diffuse thinning without endocrine abnormality; E and F, diffuse thinning and hypothyroidism.

To see whether these trends, clearly marked in selected cases, could be substantiated for all subjects, mean diameter classes were plotted for the complete data (Fig. 3). It was immediately clear that normal subjects showed a symmetrical distribution of diameters, with a marked peak at 0·08 mm. In contrast, subjects with diffuse thinning without endocrine abnormality showed a wide spread of diameters, with 2 equal peaks at 0·04 mm and 0·06 mm. The hypothyroid group showed a similar distribution, except that there was a smaller proportion of hairs in the thicker categories, and a higher peak (34% compared to 26%) at 0·04 mm.

As a final analysis, an attempt has been made to assess these differences in relation to age (Figs. 4, 5). Normal subjects (Fig. 4) showed a clear peak at 0·08 mm up to the age of 49. The secondary peak at 0·1 mm, seen in alopecia as well as normal subjects between 30 and 49 years old, may represent grey hairs, which are known to have an increased diameter. After the age of 50, the bulk of the diameters of normal subjects were evenly distributed between 0·06 mm and 0·08 mm. In diffuse thinning without endocrine abnormality a marked peak at 0·08 mm occurred only between 20 and 29 years; these subjects showed a second marked

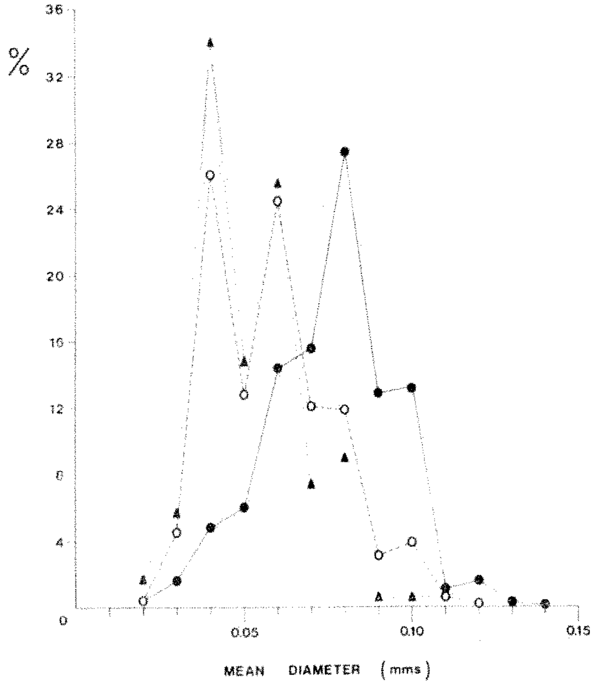

FIG. 3.—Variation in hair diameter in the 3 groups studied.———●———, normal subjects; – – –○– – –, diffuse thinning without endocrine abnormality; . . . . . .▲. . . . . ., diffuse thinning and hypothyroidism.

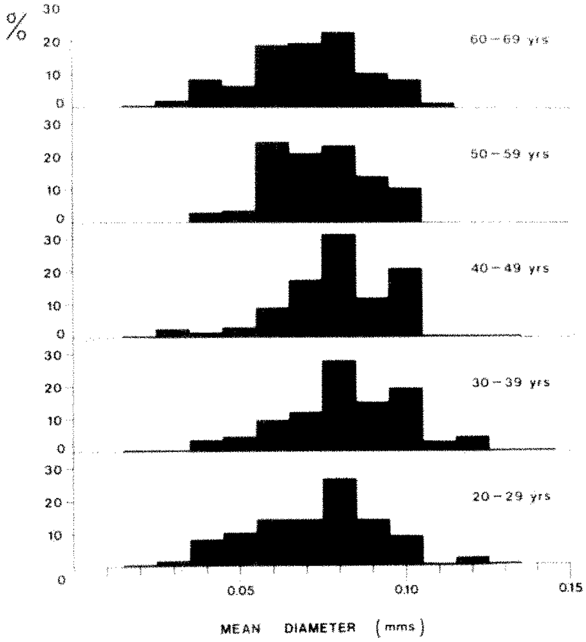

FIG. 4.—Variation in hair diameter related to age. Normal subjects.

peak at 0·04 mm. With age, the upper peak moved to 0·06 mm, while the second peak at 0·04 mm became more marked. Thus a striking feature of diffuse thinning without demonstrable endocrine abnormality appears to be the emergence of 2 predominant types of hair. Insufficient data were available to make a similar age analysis for the hypothyroid group.

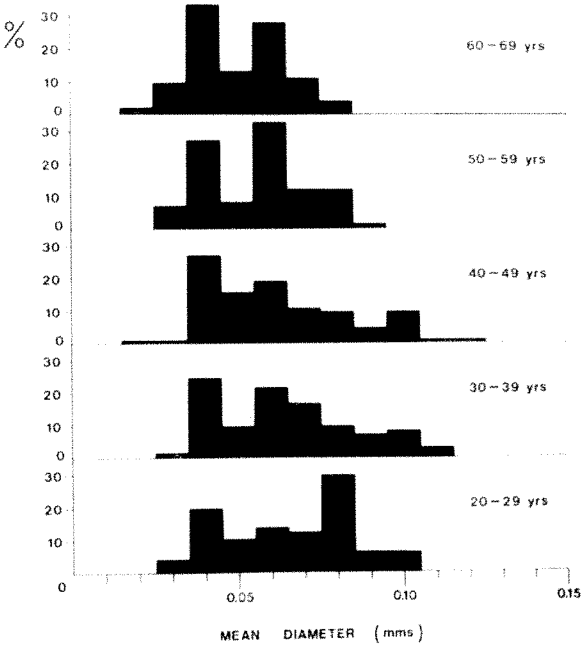

FIG. 5.—Variation in hair diameter related to age. Diffuse thinning without endocrine abnormality.

DISCUSSION

It is clear from our results that the diameter of each hair is constant throughout its length, except at the tip, and sometimes—when in telogen—near the root. Estimations of hair diameter can therefore be made on the basis of a single measurement, provided the regions adjacent to the tip and root are avoided.

The finding that there is a gradual decrease in mean hair diameter with age confirms the findings of Barman *et al.* (1965). These authors found that the proportion of thinner hairs over the whole scalp, and especially in the frontal and crown regions, increased with age in both sexes. They subsequently concluded (Barman *et al.*, 1969) that the difference between premature baldness and normal deterioration was only one of degree.

Barman *et al.* (1969) were also aware that, even in advanced male pattern baldness, some normal hairs remain in the central region of the scalp as well as elsewhere. Our finding that the hairs in female diffuse baldness differ from those of normal subjects in that they fall into 2 distinct diameter ranges lends support to the view that diffuse thinning in the female is the equivalent of, and has a similar aetiology to, pattern baldness of the male. Our evidence suggests that hypothyroid alopecia may sometimes be distinguishable in that the decrease in diameter is more severe or occurs in a greater proportion of follicles than in diffuse thinning without hypothyroidism. Taking all cases into account, however, there appears to be no diagnostic feature which could be generally applied to distinguish between familial and hypothyroid thinning. It is possible indeed that clinical thinning in hypothyroidism occurs only when the condition adds to or accelerates a familial tendency.

The demonstration that there is a wide range of hair thickness, and in particular that there are 2 separate peaks of diameter in alopecia subjects, with the implication that follicles have not all behaved identically, suggests that there may be more than one type of follicle in the human scalp. This is of particular interest because of 2 other recent findings. The first is that, in the guinea-pig—an animal which has been believed to share with man a mosaic pattern of follicular activity—it appears that there is a measure of synchrony within each follicular type, although the different types are out of phase (Jackson and Ebling, 1971). The second is that the follicles in the scalp of newborn man appear to show a wave pattern of activity, manifest because their periods are relatively short (Pecoraro *et al.*, 1964). The evidence calls into question the view that the pattern of follicular activity in the human scalp is a mosaic.

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ABNORMAL HAIR SHAFTS IN PSORIASIS ON SCANNING ELECTRON MICROSCOPY*

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Summary.—We have examined hairs from psoriatic patients by scanning electron microscopy and compared them with hairs from normal subjects. Micropits were seen in normal hair cuticle cells and in those from psoriatic subjects. Their significance is unknown. Dystrophic changes of these cells are associated with the psoriatic diathesis, occurring significantly more often in hairs from unaffected as well as from affected skin of patients with psoriasis compared with controls. We confirm previous observations that the hairs growing from psoriatic plaques are significantly thinner than those growing elsewhere on psoriatic subjects and than those of normal controls. These studies confirm and expand previous evidence of hair shaft abnormalities in psoriasis.

THERE is a good deal of evidence to suggest that psoriasis is a diffuse cutaneous disorder. The existence of the Koebner phenomenon, and epidermal turnover studies (Hell and Hodgson, 1966; Porter and Shuster, 1969) are evidence in favour of such a hypothesis. It is therefore reasonable to expect that hair follicles might also be affected, although clinical observers have almost without exception denied any such involvement of the hair shafts or follicles. Recently, however, there have been a number of observations indicating possible involvement of the hair in psoriasis (Braun-Falco and Rassner, 1966; Orfanos *et al.*, 1970; Shuster, 1971). Orfanos and his colleagues (1970) reported changes in the hair shaft on scanning electron microscopy in specimens from 2 patients with psoriasis. Shuster (1971) described an alopecia which he asserted occurred commonly in patients with psoriasis. In this paper we expand these observations on a quantitative basis in a series of patients and controls.

MATERIALS AND METHODS

Observations on normal hair have shown that the " ideal hair ", with regularly overlapping cuticular cells, is far from the usual finding, an appearance such as this often being found only very near to the root. Further along the hair one finds the development of a crenellated edge to the cuticular cells and, as this becomes more pronounced with distance from the root, one encounters areas where entire cuticular cells have disappeared, until eventually, in very long hairs, there is complete loss of cortical pattern. We have demonstrated this as a regularly progressive change in long hairs (Bottoms *et al.*, 1972). This phenomenon was also alluded to in a study of hair in alopecia areata (Jackson *et al.*, 1971). Because of these changes, probably due to wear and tear, the present study has been confined to observations made at the same site on each hair and where the hair just appears above the surface of the epidermis.

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* Based on a paper read to the British Association of Dermatology, Edinburgh, 1971.

Hairs were obtained from the scalp by rapid plucking to minimize trauma artefact (Maguire and Kligman, 1964), and from the skin elsewhere by stripping with double sided adhesive tape. They were then coated with gold in a vacuum chamber, and subsequently photographed, using a Cambridge II Stereoscan electron microscope, the root always pointing towards the left upper quadrant during observations. We have examined 87 hairs taken in this way from 36 normals (aged 18 to 52 years, mean 28·2, S.D. ± 10·4); 29 hairs from the unaffected parts of 15 patients with psoriasis (aged 7 to 74 years, mean 30·5, S.D. ± 20·9); and 61 hairs from the affected parts of 21 patients with psoriasis (aged from 7 to 71 years, mean 40·5, S.D. ± 19·9) (Table I).

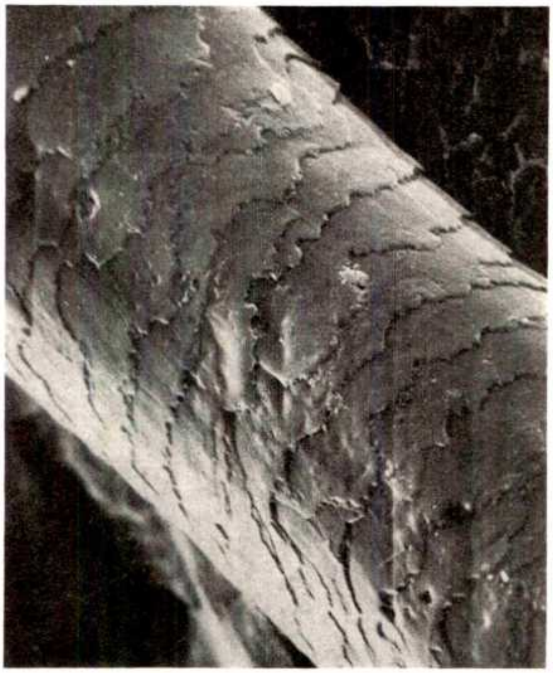

FIG. 1.—Micropits of hair cuticle cells.

TABLE I.—*Numbers of Subjects and Numbers of Hairs Studied per Subject*

Controls		Unaffected		Affected	
Number of subjects	Number of hairs	Number of subjects	Number of hairs	Number of subjects	Number of hairs
1	1	1	1	3	1
29	2	14	2	5	2
3	3	—	—	6	3
1	4	—	—	6	4
1	7	—	—	1	6
1	8	—	—	—	—

All the photographs were of standard dimensions, and the following observations were made:

1. *Micropits:* (Fig. 1) presence or absence and number per photograph.

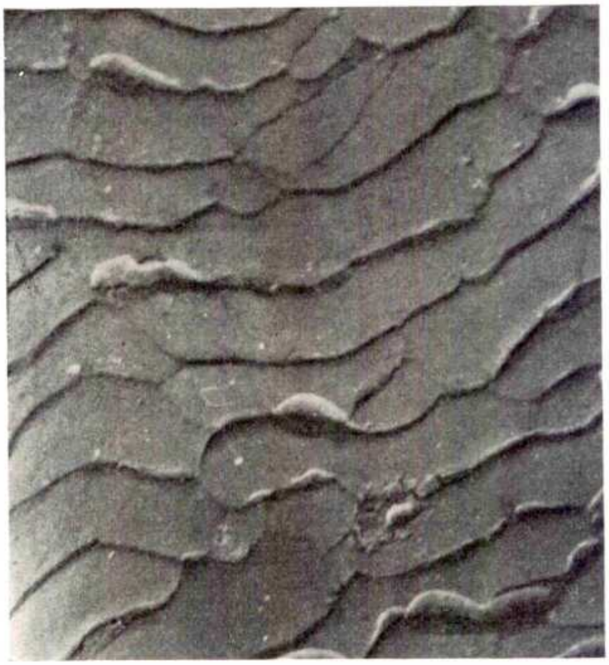

FIG. 2.—Upturned edges of hair cuticle cells.

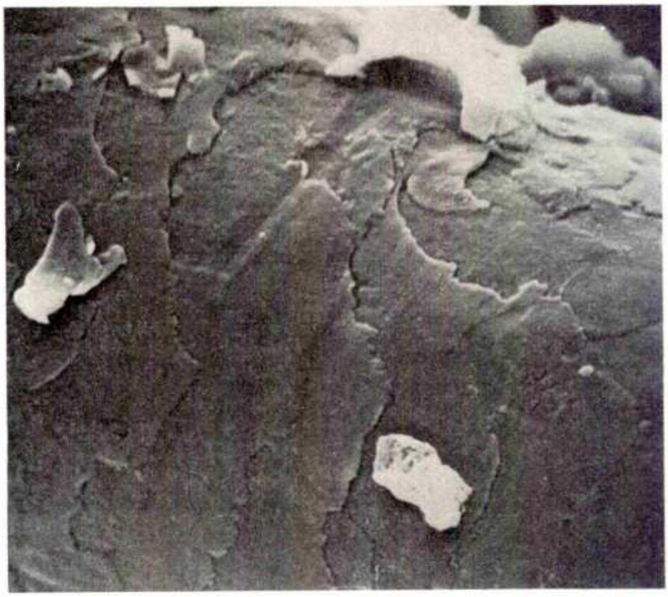

FIG. 3.—Dystrophic changes of hair cuticle cells.

2. *Upturned edges of cuticular cells:* (Fig. 2) presence or absence and number per photograph.

3. *Dystrophy*: presence or absence. It was found impracticable to devise any satisfactory method of quantifying dystrophy.

Dystrophy occurred in a minor degree (Fig. 3) as generalized roughness and raggedness of the surface, and in a more severe form (Fig. 4) where each cell was so distorted as to become unrecognizable, yet not completely torn off. We excluded from what we call dystrophy cells showing only a crenellated border. We also excluded the following artefactual appearances: small eruptions produced by focussing at high magnification, lines produced by the edges of the scanning picture, cracking produced by drying after gold coating and overlaying of the cuticular hair surface by epidermal corneocytes, or other extraneous debris.

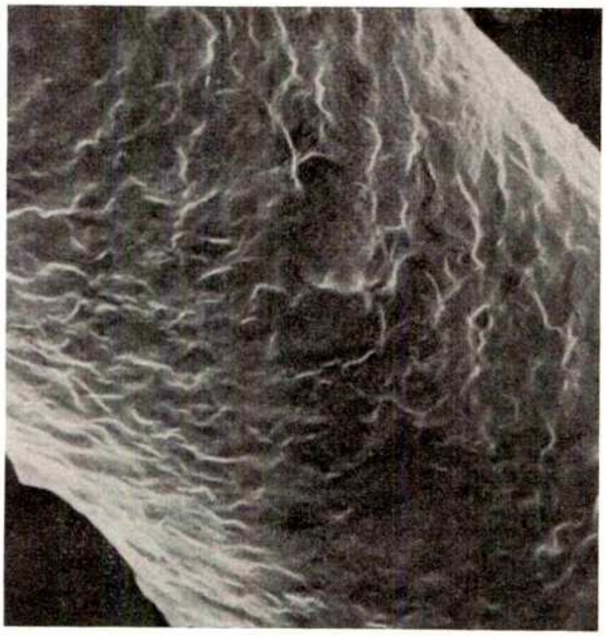

FIG. 4.—More severe dystrophic changes of hair cuticle cells.

Photographs were taken of the same site on the hair × 1000 magnification; these were read blind in a random order by 2 observers, separately, and there was significant agreement between the observers at the 0·05 level for each parameter listed. In view of this, the results of each observer are pooled in the tables given below. A random sample of photographs from 53 normal, 14 unaffected psoriatic and 41 affected psoriatic hairs was reexamined after 4 months. After this interval there was significant agreement with the previous readings on the presence of micropits, upturned edges, and dystrophy ($P < 0{\cdot}05$). Hair diameters were calculated from measurements made on each photograph of scalp hairs, correcting for actual lens magnification.

RESULTS

Micropits.—Table II shows that, although the presence or absence of pits does not correlate with the diagnosis of psoriasis (II(a)), when the data is analysed for

TABLE II (a).—*Presence or Absence of Pits*

	Controls	Unaffected	Affected
Pits present	85 (49%)	29 (50%)	57 (47%)
Pits absent	89 (51%)	29 (50%)	65 (53%)

Analysis: Control: unaffected χ^2, 1df $= 0{\cdot}02\ P > 0{\cdot}90$.
Control: affected χ^2, 1df $= 0{\cdot}13\ P > 0{\cdot}75$.

TABLE II (b).—*Number of Pits per Standard Area*

Number of Pits	Controls	Unaffected	Affected
0	89 (51%)	29 (50%)	65 (53%)
1	19 (11%)	8 (14%)	6 (5%)
2	17 (10%)	6 (10%)	16 (13%)
3	14 (8%)	7 (12%)	19 (16%)
4+	35 (20%)	8 (14%)	16 (13%)

Analysis: Control: unaffected χ^2, 4df $= 2{\cdot}17\ P > 0{\cdot}70$.
Control: affected χ^2, 4df $= 9{\cdot}49 = 0{\cdot}05$.

number of pits per standard area (II(b)), there is a difference between control hairs and hairs from psoriatic plaques significant at the 5% level.

TABLE III (a).—*Upturned Edges: Presence or Absence*

	Controls	Unaffected	Affected
Upturned edges	87 (50%)	24 (41%)	56 (46%)
Upturned edges absent	87 (50%)	34 (59%)	66 (54%)

Analysis: Control: unaffected χ^2, 1df $= 1{\cdot}30\ P > 0{\cdot}2$.
Control: affected χ^2, 1df $= 0{\cdot}48\ P > 0{\cdot}3$.

Upturned edges.—Table III indicates that there is no correlation between the presence of upturned edges and psoriasis.

TABLE III (b).—*Numbers of Upturned Edges per Standard Area*

Numbers of upturned edges	Controls	Unaffected	Affected
0	87 (50%)	34 (59%)	66 (54%)
1	18 (10%)	7 (12%)	9 (7%)
2	13 (8%)	8 (14%)	10 (8%)
3	22 (13%)	2 (3%)	5 (4%)
4+	34 (20%)	7 (12%)	32 (26%)

Analysis: Control: unaffected χ^2, 4df $= 7{\cdot}6\ P > 0{\cdot}10$.
Control: affected χ^2, 4df $= 8{\cdot}2\ P > 0{\cdot}05$.

Dystrophy.—Table IV shows that, in hairs from both psoriatic plaques and unaffected skin of psoriatics, dystrophic changes occur significantly more often than in controls.

TABLE IV

	Controls	Unaffected	Affected
Dystrophy present	36 (21%)	21 (36%)	48 (39%)
Dystrophy absent	138 (79%)	37 (64%)	74 (61%)

Analysis: Control: unaffected $\chi^2 = 5{\cdot}6\ P < 0{\cdot}025$.
Control: affected $\chi^2 = 12{\cdot}3\ P < 0{\cdot}01$.

TABLE V

Mean Hair Diameter (μm) (±S.D.)

	Psoriasis	
Normal	Unaffected areas	Plaques
70·2 ±12·3	66·8 ±11·6	55·2 ±14·6

Hair diameter (Table V).—Hairs from psoriatic plaques were significantly narrower than those taken from controls ($t = 4{\cdot}96$, $P < 0{\cdot}001$). There was no significant difference between the diameter of control hairs and those taken from the unaffected areas of psoriatics ($t = 0{\cdot}74$, $P > 0{\cdot}1$). A comparison of affected and unaffected areas of psoriatics did show a significant difference ($t = 2{\cdot}06$, $P < 0{\cdot}05$), the hairs from affected areas being narrower.

DISCUSSION

The micropits that we describe have not to our knowledge been previously noted in normal hair; but, as with upturned edges, this study shows that their presence or absence bears no clear relation to the presence or absence of psoriasis at the site from which they were plucked.

Our finding that dystrophic changes in the hair shafts occur significantly more often in psoriatics than controls is of great theoretical interest, particularly since we have demonstrated that this abnormality occurs almost as frequently in hairs from apparently normal skin as in hairs from psoriatic plaques.

The hair diameter measurements support the idea of psoriatic alopecia (Shuster, 1971).

Mr E. H. Boult, the School of Chemistry, University of Newcastle-upon-Tyne, provided facilities for this study. Mrs D. Weightman, of the Department of Medical Statistics, helped considerably with the statistical analysis. We also wish to thank Professor Sam Shuster for advice and Mr H. Sargeant for invaluable technical assistance.

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Case Report

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POST KALA-AZAR DERMAL LEISHMANIASIS

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Summary.—A case of post kala-azar dermal leishmaniasis is reported to illustrate the late onset of the dermal changes and the difficulties of diagnosis.

VISCERAL leishmaniasis (kala-azar) is a disease seldom met with in British clinical practice, and post kala-azar dermal leishmaniasis (P.K.D.L.) is even less commonly seen, although the influx of immigrants from endemic areas and the increasing frequency of travel to such areas makes their recognition important (Shortt, 1947; Ecker and Lubitz, 1947). The following case history illustrates some of the difficulties encountered in establishing the diagnosis, in particular the ease with which the condition may be mistaken for other diseases, and also the unusually long incubation period of kala-azar and late onset of the dermal changes.

CASE REPORT

A business man aged 52 was first seen in July 1969, complaining of thickening of the skin of his face, and a rash on his face, neck, arms and trunk.

He had noticed red patches on his back and arms in 1955 whilst sunbathing and swimming. This embarrassed him to such an extent that he gave up swimming in public. In 1966 the patches had spread to his hands and face, and thickening of the skin of his face and broadening of his nose were evident.

He had lived a considerable part of his life abroad, and saw active service during World War II in North Africa, Burma and India. From January 1947 to July 1948 he lived in the Dum-Dum region of Calcutta before moving to Lahore in West Pakistan. In 1956 he returned to Britain for 7 years until 1963 when he left to work in Nigeria where he has remained.

His past medical history revealed that he was fit until 1950. Whilst in West Pakistan he contracted an intermittent febrile illness. During the afebrile period he felt well and was able to work. A diagnosis of malaria was made and he was treated

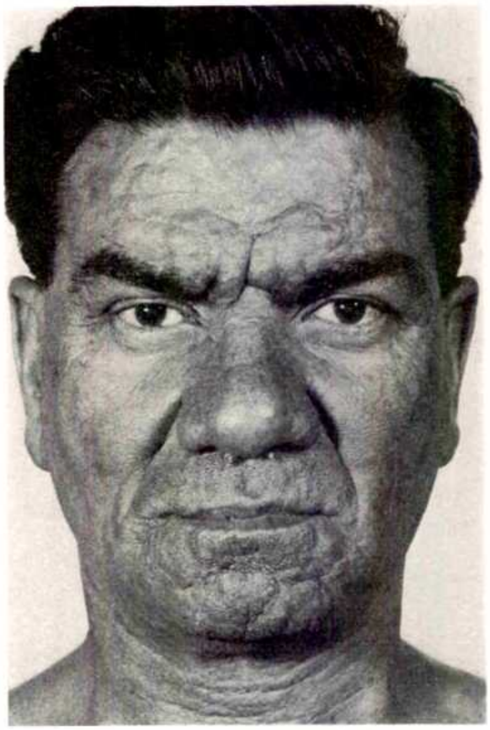

FIG. 1.—Nodular eruption on the face.

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accordingly but without result. Six months later the diagnosis of kala-azar was established, and he responded rapidly to therapy.

On examination he had an indurated nodular eruption affecting his forehead, nose, lips, chin and neck (Fig. 1). Most of the indurated skin was of normal colour, but some areas were a little erythematous, with either increased or decreased pigmentation of mild degree. Prominent nodules were present on the vermilion of his upper lip and on the edges of his nostrils. The skin of his superior orbital ridges and glabellar region was particularly thickened, giving him a peculiar frowning appearance.

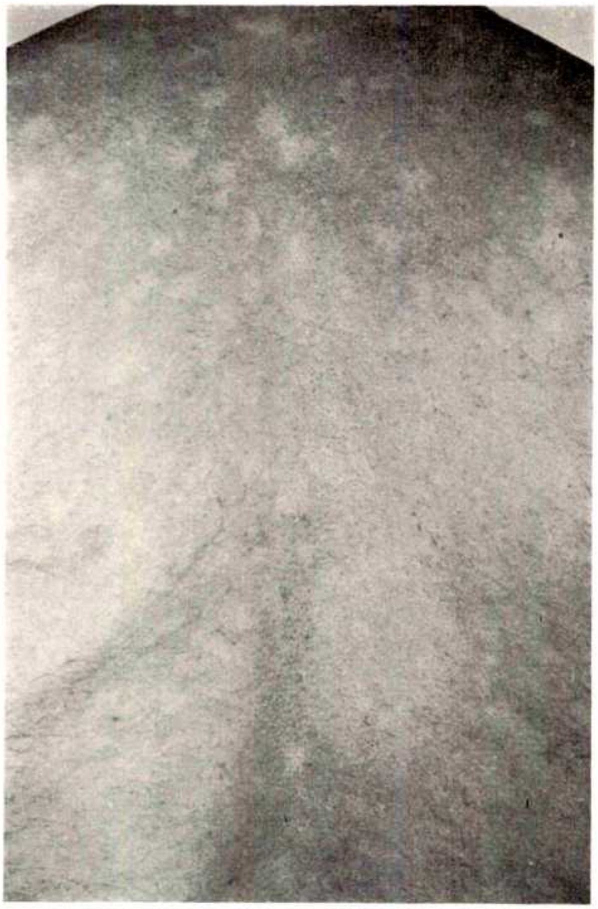

FIG 2.—Hypopigmentation over the back.

In addition he had macules, some of which were hypopigmented, on his back, chest and arms (Fig. 2). One of us (D.D.M.) found minimal impairment of sensation to temperature and light touch over some of these macules. Full general examination revealed no abnormality, and his liver and spleen were not palpable.

Investigations

The Hb was 14·6 g%, and the E.S.R. was normal. The W.B.C. count and differential, blood urea, liver function tests, Wassermann test, microscopy of the urine, and chest

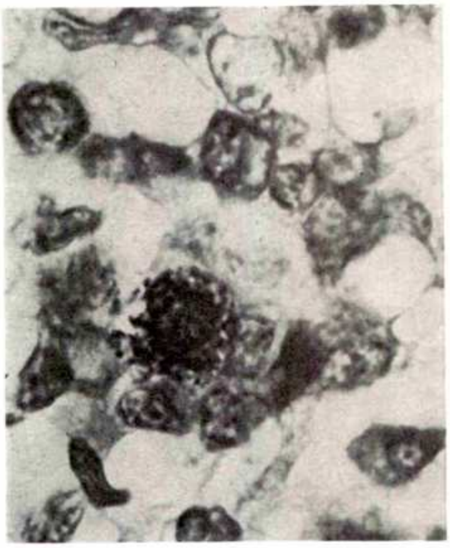

FIG 3.—A mass of Leishman–Donovan bodies within a macrophage. (Giemsa, × 1500.)

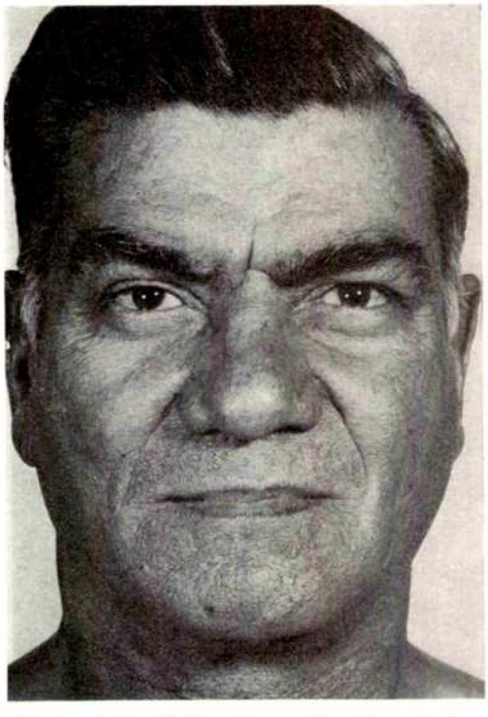

FIG 4.—Patient's face 18 months after starting treatment.

radiograph were also normal. The serum proteins showed normal total levels, but slightly raised γ-globulins.

Initially, skin biopsies were taken from the forehead and back. They showed a dense infiltration of the dermis with chronic inflammatory cells, including plasma cells and lymphocytes. The nerve bundles were not involved. It was not until the first diagnosis of leonine leprosy was ultimately disproved by the failure to demonstrate leprosy bacilli that leishmaniasis was considered. Another biopsy was taken from the patient, this time from the vermilion of his upper lip. It was then that Leishman–Donovan bodies were seen in large numbers (Fig. 3). Review of the first sections revealed sparse Leishman–Donovan bodies in the macrophages.

Skin tests showed: (1) A weakly positive response to 100 μg of *L. donovani* protein antigen; (2) No response to 100 times the standard strength leishmanin or to 100 μg of *L. tropica* protein antigen; (3) A positive response to *L. donovani* but not to *L. tropica*, using lymphocyte transformation studies.

These tests show fairly conclusively that the Leishman–Donovan bodies present in the skin were *L. donovani* (the cause of kala-azar and P.K.D.L.) and not *L. tropica* (the cause of cutaneous leishmaniasis in the Old World). It is interesting that skin testing with *L. donovani* antigen in kala-azar gives a negative result; it is only after the illness that it becomes positive and therefore helpful in P.K.D.L.

Treatment and progress

The patient was treated with sodium antimony gluconate (Pentostam) in a dose of 6–8 ml daily by intravenous injection until a total dosage of 100 ml was given. The injections would normally have been given twice weekly, but the patient demanded that the treatment be completed in the shortest possible time.

Within 2 weeks of starting therapy the indurated consistency of the skin had begun to soften; and, by the end of the first month, there was a marked reduction in the thickened appearance of the face. This improvement continued gradually, as illustrated by the accompanying photographs (Fig. 4, 5) taken 18 months after treatment was instituted. A skin biopsy was performed after the fourteenth injection of Pentostam, and no Leishman–Donovan bodies were found. Skin smears taken at the same time by the scraped-incision method, as used in the diagnosis of leprosy, were also negative for Leishman–Donovan bodies, whereas they had been positive prior to treatment.

DISCUSSION

The patient almost certainly acquired kala-azar in 1947 whilst residing in Dum-Dum, which is 8 miles north-east of Calcutta. This region of India is an endemic area of kala-azar, whereas West Pakistan, where he went in 1948, is not. In fact, " Dum-Dum fever " was the original name given to kala-azar. The fact that he did not develop symptoms of kala-azar until 1950, nearly 3 years later, is quite in keeping (Stone *et al.*, 1952; Most and Lavietes, 1947; Jopling, 1955), although shorter incubation periods are normally quoted (Most and Lavietes, 1947; Ecker and Lubitz, 1947).

An initial diagnosis of malaria is a common clinical error (Stone *et al.*, 1952; Most and Lavietes, 1947; Brahmachari, 1923) which has to be revised if the malarial parasite is not found in the blood and the patient fails to respond to treatment. Absence of malaise, even during febrile phases, is another differentiation from malaria, and the fact that our patient was able to go to work throughout the greater part of his illness is characteristic of kala-azar.

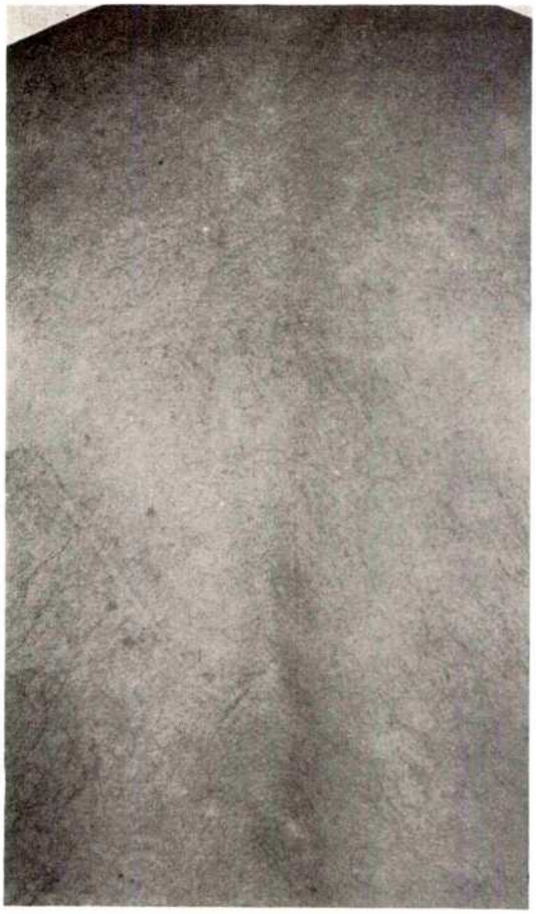

FIG 5.—Patient's back 18 months after treatment, showing repigmentation.

Post kala-azar dermal leishmaniasis was first described in 1922 by Brahmachari (1922). Three types of skin lesion were described—hypopigmented macules on the trunk and limbs, which increase in size and coalesce; a photosensitive erythema in a " butterfly " distribution on the face; and papillomatous nodules on the chin, nose and cheeks, " somewhat resembling small leprotic nodules " (Brahmachari, 1923). Leishman–Donovan bodies were also demonstrated in the skin nodules (Brahmachari 1923, 1934). In 1927 Das Gupta (Napier and Das Gupta, 1930) inoculated mice with material from a dermal lesion. He produced symptoms similar to those produced by inoculation from a case of kala-azar, thus providing experimental evidence of the relation between the 2 conditions.

Dermal involvement occurs in about 6% of patients after they have contracted kala-azar in India, but is not seen in the Mediterranean variety, and is rarely encountered in South America. The normal time interval before the appearance of skin lesions is one to 2 years (Acton and Napier, 1927; Napier and Das Gupta, 1930). This case was unusual in that the patient did not remark on the skin changes until some 5 years after the original infection. This, coupled with the fact that the patient failed to seek medical attention concerning his skin until almost 15 years later, made the diagnosis far from obvious. The confusion with leprosy is classical (Brahmachari, 1934). The thickened skin on the face suggested the leonine facies of lepromatous leprosy, and the hypopigmented macules on the trunk were much in keeping with the diagnosis of leprosy. These were also found to be minimally anaesthetic, and seemed to support the diagnosis, but this was later discounted, for minimal anaesthesia is a misleading finding in a wide range of dermatoses in which infiltration with neoplastic or chronic inflammatory cells is a feature.

Two useful clinical guiding points in excluding lepromatous leprosy are, firstly, the absence of induration of the ears (Muir, 1930), and secondly, the absence of thinned eyebrows, as was the case here; and, when our investigations for leprosy proved negative, the diagnosis of post kala-azar dermal leishmaniasis was considered and found to be correct.

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Pharmacology and Treatment

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ULTRAVIOLET THERAPY OF PSORIASIS: A CRITICAL STUDY

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Summary.—By means of the method of paired comparisons in 33 patients, the value of the treatment of psoriasis by u.v. irradiation, especially in combination with coal tar, is analysed. In all cases u.v. light failed to cause any demonstrable therapeutic effect.

Ultraviolet light has long been used in the treatment of psoriasis. It was introduced by Goeckerman (1925) because of the observation that, in most patients, psoriasis is better in summer than in winter and also because it is believed that a sunny climate has a favourable effect.

Goeckerman tried to enhance the effect of sun by artificial sensitization of the affected parts of the skin by various fluorescent substances; some of them were known for their photosensitizing properties. All these substances failed. He next tried the use of crude coal tar, which, according to him, at once appeared to have a clear photosensitizing effect.

After these experiments a scheme of treatment was introduced by Goeckerman in which, in the afternoon or in the evening, the following ointment is applied to the skin: crude coal tar 2–6, zinc oxide 30, soft paraffin to 120. The next day the remaining ointment is removed with olive oil, and only a thin film, sufficient to leave a brown colour on the skin, is left purposely. These stained patches are then irradiated with a mercury quartz lamp. To avoid irritation, after an initial dose of 1 min at a distance of 75 cm, the time of exposure is gradually lengthened.

Goeckerman is of the opinion that it is not necessary to provoke a strong erythema but that a gradually increasing pigmentation should be the objective. After irradiation, the patient takes a bath with soap and water. The ointment is reapplied only after an interval of several hours.

Since the description of this method in 1925 it has been used all over the world, both modified and unmodified.

Goeckerman (1931) himself claimed good results in 178 of 181 patients treated in this way. Mild cases cleared in 2–3 weeks, and severe cases in 3–4 weeks. Rossi-Soffar (1965), using a slightly modified method in which 10% coal tar is used and u.v. irradiation is given after a tar bath and not before the bath as Goeckerman did, claims a mean treatment period of 21·46 days in 88 patients. This does not differ essentially from the result of treatment with chrysarobin. MacKenna (1959) also uses a modified method in which 4–10% coal tar paste BPC

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is used, and a tar bath instead of a bath with soap and water. It is claimed that most patients clear within 21 days. Ellis *et al.* (1948) used undiluted liquor picis carbonis instead of an ointment with crude coal tar. They could not demonstrate, however, an effect of u.v. irradiation additional to that of tar. Church (1958) used a combination of coal tar and ammoniated mercury in an ointment base under a stockinette suit, together with tar baths and u.v. irradiation. Only 4·6% of 260 patients did not react to this treatment. According to Perry *et al.* (1968) wood tar or ichthyol can also be used in combination with u.v. irradiation.

Beside combinations of u.v. light and tar therapy, u.v. irradiation as such is recommended, but Epstein (1947) did not observe favourable results from this treatment in a series of 9 patients. In a statistical study Bowers *et al.* (1966) came to the conclusion that dithranol ($\frac{1}{2}$% in Ingram ointment base) and u.v. light are of about equal value in the treatment of psoriasis, but that the combination of these has a still better effect. This combination is also advised by Ingram (1953), whose treatment consists of dithranol in a special base, daily coal tar baths and u.v. irradiation.

The combination of u.v. light and chrysarobin was recommended by Maschkilleisson and Abramowitsch (1934) in a controlled study in which they found less irritation and a better antipsoriatic effect when chrysarobin therapy was combined with u.v. irradiation.

Few data are to be found in the literature on the theoretical background of u.v. therapy in psoriasis. According to Ippen (1966/67) u.v. B (280–315 nm) is the active part of the spectrum in psoriasis. Its mode of action on the epidermis appeared to be about the same as that of antipsoriatic anthracene derivatives. Bowers *et al.* (1966) mention wavelengths below 320 nm as the therapeutically active part of the u.v. spectrum.

As to photosensitization by coal tar, Foerster and Schwartz (1939) state that the action spectrum of coal tar is between 330 and 550 nm. Crow *et al* (1961) found abnormal spectral reactivity after exposure to pitch in the region 340–430 nm. With pure liquor picis carbonis they obtained photosensitivity at wavelengths between 340 and 400 nm. The experiment with crude coal tar, however, was entirely negative. Frank (1949) could not demonstrate photosensitization under the conditions recommended by Goeckerman (1925, 1931) for his treatment. The experiments of Frank (1949) are supported by an Editorial Comment in the *Yearbook of Dermatology and Syphilology* (1949). The reasons for combining chrysarobin (and dithranol) with u.v. light are discussed by Maschkilleisson and Abramowitsch (1934).

In spite of the frequent use of u.v. treatment in psoriasis, mostly in combination with coal tar (Goeckerman treatment) or other tar preparations, or alone, or in combination with dithranol or chrysarobin, controlled studies of the exact part played by u.v. light in such treatment routines are almost entirely lacking. Only the work of Ellis *et al.* (1948), Bowers *et al.* (1966) and Maschkilleisson and Abramowitsch (1934) deserves mention here; and, as far as the author knows, such an investigation has never been performed by the method of paired comparisons, which is considered by far the best method to investigate the effect of external treatment (Siemens, 1942, 1943); von Czetsch-Lindenwald and Schmidt-La Baume, 1950). We therefore tried to carry out such an investigation. We had previously demonstrated by the same method the effect of coal tar and dithranol in the treatment of psoriasis (Young, 1970a, 1970b). In the latter part of this work, however, we found u.v. irradiation in general not essential to the success of the Ingram scheme of dithranol treatment.

In the present study of the effect of u.v. light we confined ourselves to the effect of a conventional u.v. lamp because this had been used by all other authors.

MATERIALS AND METHODS

Thirty-three patients were treated for at least 14 days by means of the method of paired comparisons; the results were statistically evaluated. For details of this method see Young (1970a). These 33 patients were divided into 3 groups:

(a) A group of 10 patients in which u.v. irradiation was given to one side of the body, the other side remaining untreated.

(b) A group of 13 patients in which the original Goeckerman treatment (see above) to one side of the body was compared with the same treatment without u.v. irradiation to the other side.

(c) A group of 10 patients in which a modified Goeckerman treatment (see below) was compared with the same treatment without u.v. irradiation.

The part of the body not irradiated was always covered with a thick linen sheet during irradiation of the other part. For all irradiation the Philips " Biosol " 500 W u.v. lamp was employed: this is a high pressure mercury arc with a high output in the sunburn wavelengths down to 275 nm.

In Groups (a) and (b) irradiation was performed as in the scheme of Goeckerman (see above) to avoid irritation.

In Group (c) a modified Goeckerman treatment was employed. In this modified scheme patients were treated with 10% crude coal tar in zinc paste.

In the morning the patients first took a bath with soap and water and then were given u.v. irradiation in a gradually increasing dose. In the evening the ointment was applied again.

RESULTS

Groups (a), (b), (c). In none of the 3 trials could any difference be demonstrated between the response of the irradiated and that of the unirradiated side. It is concluded that, under the conditions of these trials, u.v. irradiation has no significant effect.

DISCUSSION

In a controlled study of 33 patients we could not demonstrate any effect of u.v. therapy, either alone or in combination with coal tar. Thus, in our case, u.v. therapy appeared to have no value in the treatment of psoriasis; certainly no direct effect upon the irradiated skin was demonstrable. It could be objected, however, that the irradiation of part of the body could have some beneficial effect also on other parts of the skin. This seems very unlikely to us, because we always worked with treatment schemes of 6 fields (Young, 1970a); only one of them received u.v. irradiation, for example the left arm, when the right arm was used as the control. The other 4 fields received quite different treatment. We think that, under such circumstances, any influence of the irradiated field upon its contralateral control is very improbable.

Reviewing critically the literature on this subject, one must conclude that, although the good results of combined treatment, especially with tar and u.v. light, are nearly always ascribed, at least in part, to the favourable effect of the irradiation, no proof of this effect has been provided, since no comparisons were made with the effect of tar alone (Epstein, 1947; Goeckerman, 1925, 1931; Forck, 1966/67; MacKenna, 1959; Church, 1958).

Only Ellis *et al.* (1948) made a comparison between the effect of liquor picis carbonis in combination with u.v. irradiation and liquor picis carbonis alone, treating 2 groups of 16 and 24 patients respectively. They found no difference between the response of these 2 groups, which suggests that the active agent is the tar rather than the u.v. light.

One may conclude, therefore, that there is no experimental justification for the use of u.v. light, or its combination, especially with coal tar, in the treatment of psoriasis.

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GRISEOFULVIN THERAPY IN LICHEN PLANUS

A DOUBLE-BLIND CONTROLLED TRIAL

V. N. SEHGAL, G. J. S. ABRAHAM, AND
GAURI BAZAZ MALIK

Summary.—Thirty-four patients with lichen planus were treated in a double-blind trial in which either 500 mg of griseofulvin-FP or a placebo was issued to the patients on a random distribution basis. A significant difference was found between the results obtained with griseofulvin and those with the placebo. The favourable response to the treatment was accompanied by more or less typical histopathological features. No untoward effects, or recurrence in lesions were noted in the course of the trial.

LICHEN planus is a well recognized clinical entity of unknown aetiology (Samman, 1968; Sehgal *et al.*, 1972). The results of the treatment of this condition are generally unsatisfactory (Samman, 1968). Encouraged by the promising results obtained by griseofulvin therapy in an earlier pilot trial (Sehgal *et al.*, 1971) a double-blind controlled study was undertaken to assess the usefulness of this drug in lichen planus. The observations made during the course of the trial are reported in the present paper.

MATERIAL AND METHODS

Thirty-eight patients with lichen planus were initially included in the study. The diagnosis in each case was made on the clinical morphology of lesions. The clinical impressions in all cases were confirmed histopathologically (Sehgal *et al.*, 1972). Four of the 38 patients failed to carry out the treatment completely, so that the data on 34 patients only are available, 19 being females and 15 males. The youngest patient was 10 years of age, while the oldest was 55 years. The duration of the disease was variable; some cases were of recent onset, while the others were of long duration.

Each patient was given 4 capsules daily in accordance with a randomization schedule, and neither the investigator nor the patient knew the contents of the capsules. The capsules contained either 125 mg of griseofulvin-FP or a placebo. The trial extended from July 1971 to March 1972. The patients were followed by the senior investigator every week, from the start of the trial. In the majority of cases the trial was continued for 4 weeks, but, in two cases, the duration was increased to 6 weeks. The assessment of improvement in the lesions of lichen planus was made on the basis of gradual reduction of itching and scaling, and flattening of the lesions to leave post-inflammatory pigmentation. Further, in 10 of the 18 cases, where a good response to the treatment was noticed, a follow-up histopathological examination was made. A serial photographic record of clinical improvement of the condition was also maintained.

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Requests for reprints should be addressed to Professor Sehgal, Department of Venereology and Dermatology.

RESULTS

The analysis of data after decoding is shown in Table I. Eighteen (52·9 %) of the 34 patients showed complete regression of skin lesions, while 16 (47·1 %) showed no response. Further, 12 (70·6 %) patients who were on griseofulvin-FP showed regression, which was evident by reduction in itching, and by peeling and flattening of lesions. The post-inflammatory pigmentation after regression of lesions was a common feature in all patients. No response to the treatment in 5 (29·4 %) patients was seen amongst those receiving griseofulvin. In the placebo group, on the other hand, only 6 (35·3 %) patients responded favourably to treatment, while 11 (64·7 %) showed no improvement. The overall percentage response in the 2 groups is shown in Fig. 1. Statistically, the response in the 2 groups differs significantly ($\chi^2 = 4{\cdot}25$; $df = 1$; $P < 0{\cdot}05$).

TABLE I.—*Analysis of Data*

	No.	Griseofulvin percentage	No.	Placebo percentage	No.	Total percentage
Complete Regression	12	70·6	6	35·3	18	52·9
No improvement	5	29·4	11	64·7	16	47·1
Total	17	100·0	17	100·0	34	100·0

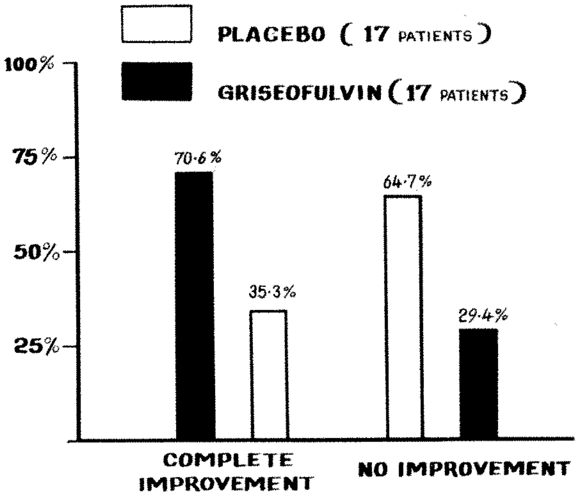

FIG. 1.—Percentage response in treated and untreated patients.

Ten of the 18 patients who showed a good therapeutic response were studied histologically. Eight of the 10 patients were receiving griseofulvin-FP and 2 the placebo. Histopathological changes were uniformly similar. They were marked by gradual decrease in the thickness of stratum corneum and stratum granulosum. The prickle cell layer was characterized by decrease in thickness, resulting in flattening of rete ridges. No significant changes in the basal cell layer were seen. The band-like infiltration in the papillary layer of the dermis showed a variable degree of reduction in chronic inflammatory cells. The 2 patients receiving the placebo revealed more or less similar changes. No recurrences of skin or mucous membrane lesions have been noticed in any of the treated cases during the course of the trial to date.

DISCUSSION

In the present trial, griseofulvin has been shown to be significantly better than a placebo in the treatment of lichen planus. These findings confirm the only earlier assessment of this drug by Sehgal *et al.* (1971).

The dose required is 500 mg of griseofulvin-FP daily in divided dosage for an optimum period of 4 weeks. The response to this drug therapy is more or less typical. It is characterized by a gradual decrease in itching, and by peeling and flattening of skin lesions. The complete regression of the lesion is marked by pigmentation at that site, which gradually disappears in a variable time period. Similar but mild changes are observed in the lesions of mucous membranes.

The serial histological studies in the course of treatment show changes as described above which go hand in hand with the morphological changes seen clinically.

The generally favourable response to griseofulvin therapy seems promising. The absence of untoward effects and of recurrences of the lesions further stresses the need for further investigation of this drug in lichen planus.

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Comment

RECENT PROGRESS IN THE FORMULATION OF VEHICLES FOR TOPICAL APPLICATIONS

In recent years, increasing attention has been paid to the effects of pharmaceutical formulation on drug activity. It is now known that substances added as excipients to pharmaceutical preparations and other factors such as the physical form of the drug can affect the rate at which drugs are released and materially modify their absorption and action in the body. The study of biological availability now plays an essential part in the development of pharmaceutical products.

The influence of the formulation on drugs applied topically was probably appreciated before the importance of biological availability was more generally realized. For a long time it has been an accepted principle that a dermatological vehicle must be compatible with the medicament and release it in an active form on the skin surface. Interest in bio-pharmaceutics, however, has stimulated the investigation of problems concerned with the formulation of vehicles for use in dermatology and their effect on the activity of the medicament. The drug-vehicle factors likely to be of importance in the formulation of topical preparations have been enumerated by Poulsen (1970) as follows:

(1) Effects of vehicles on the integrity of the skin barrier or on skin permeability.
(2) Particle size of poorly soluble suspended drugs.
(3) Chemical nature of the drug.
(4) Partition coefficient of the drug between the skin and vehicle.
(5) Viscosity of the vehicle.
(6) Drug concentration in the vehicle.

The introduction of topical corticosteroids and the development of the vasoconstriction assay (McKenzie and Stoughton, 1962) has added greatly to the study of vehicle effects on drug activity. The vasoconstriction assay was modified by Sarkany *et al.* (1965) in an experimental and clinical study of the role of vehicles in the percutaneous absorption of corticosteroids. It was shown empirically that modification of the vehicle could influence the topical activity of hydrocortisone and that percutaneous absorption was enhanced by solvents such as tetrahydrofurfuryl alcohol and dimethylacetamide. Barrett *et al.* (1965) also showed that when fluocinolone acetonide was suspended in the vehicle, reduction of particle size enhanced absorption. The absorption was further enhanced if the fluocinolone acetonide was dissolved in propylene glycol before dispersion in the vehicle (Senior, 1971). Busse *et al.* (1969) reported a similar finding for the release of betamethasone 17-valerate from ointments and Pepler *et al.* (1971) found that preparations containing betamethasone 17-benzoate in bases containing white soft paraffin and propylene glycol or white soft paraffin and isopropyl myristate gave significantly higher total scores in a vasoconstriction assay than five other ointments tested. A more accurate method of using the vasoconstrictor response to measure the influence of vehicles on the topical activity of corticosteroids was developed by Moore Robinson and Christie (1970) and Place *et al.* (1970).

The effects of polythene occlusion on the penetration of corticosteroids has been convincingly demonstrated experimentally (McKenzie and Stoughton, 1962) and exploited clinically. It is possible that the vehicle in which a drug is formulated may also influence its penetration by modifying the degree of hydration of the skin (Sarkany and Hadgraft, 1969). By measuring the effect on transepidermal water diffusion on the skin, Baker (1969) compared the occlusivity of a number of commonly used dermatological vehicles. Paraffin bases were shown to have the greatest effect in suppressing transepidermal water diffusion whereas other standard vehicles affected it to a lesser degree. Rather surprisingly, anhydrous wool fat was found to have no effect on water loss from the skin surface. The ability of paraffin-type bases to produce a degree of occlusivity has also been noted by Coldman *et al.* (1971a). It was further suggested that this might account for the placebo responses observed in assessing corticosteroid activity by the vasoconstriction test (Coldman and Lockerbie, 1971).

Other vehicles which have been used for the topical application of drugs may affect percutaneous absorption by modifying or damaging the barrier layer. Dimethylsulphoxide is the outstanding example of this type of vehicle and its use both as a therapeutic agent and as an enhancer of percutaneous absorption has been reviewed by Kligman (1965) and by Katz and Poulsen (1971). Baker (1969) found that dimethylsulphoxide increased the rate of transepidermal water loss indicating that it had a direct effect on the barrier function of the skin. Tetrahydrofurfuryl alcohol, dimethylacetamide, dimethylformamide and propylene glycol also increased the water loss from the skin but to a lesser degree. Ostrenga *et al.* (1971c) have shown that at moderate concentrations, propylene glycol has no apparent effect on the permeability of the skin but above 50% the results suggest that the permeability of the barrier is in fact decreased.

Preparations containing dimethylsulphoxide for clinical use have not been developed commercially following American reports of toxicity involving lens changes in the eyes of experimental animals. However, if used under careful clinical supervision, it appears to be a safe and effective vehicle for the application of idoxuridine in the treatment of herpetic skin lesions (Juel Jensen, 1969).

Propylene glycol has been widely used as a solvent for topical applications and has produced varying results with different drugs. In an investigation of the rate of absorption of methyl nicotinate from four different solutions, Fountain *et al.* (1969) found that absorption from propylene glycol was slower but more prolonged than from water or tetrahydrofurfuryl alcohol. It is an effective solvent for the application of fluorouracil (Eaglstein *et al.*, 1970) and in combination with ethanol for the application of retinoic acid in acne vulgaris (Kligman *et al.*, 1969; Pedace and Stoughton, 1971; Peachey and Conner, 1971). On the other hand, Burton and Shuster (1972) found that tetraynoic acid in a solution containing 20% propylene glycol in ethanol failed to produce significant reduction in sebum excretion whereas a previous study had shown a slight but significant decrease in excretion rate when tetraynoic acid was applied in simple ethanol solution (Burton and Shuster, 1970).

The varying effect of propylene glycol on the penetration of different drugs is probably due to their different solubilities. A number of studies have shown that penetration is greatest when the drug is present at its maximal solubility concentration. Below this level, the affinity of the solvent for the drug may be greater than that of the skin and penetration will be prevented. Demski *et al.* (1969) have suggested that the release of substances from topical bases is a function of their solubility in both the base and its surrounding media. For a drug to be released into a surrounding aqueous medium, it must be soluble in a non-aqueous base but not so soluble as to remain preferentially in the vehicle. Similar considerations apply to the release of drugs from aqueous vehicles into a lipid phase. Coldman *et al.* (1969) demonstrated an enhancement of percutaneous absorption of fluocinolone acetonide when it was dissolved in a mixed volatile : non-volatile solvent system. On the skin surface, the volatile constituent evaporated leaving a supersaturated solution of the corticosteroid from which penetration was maximal.

Useful information on the release of corticosteroids from aqueous propylene glycol gels has been gained by employing a model system in which isopropyl myristate was used as a receptor phase (Poulsen *et al.*, 1968). It was shown that the release of fluocinolone acetonide and fluocinonide was maximal when the formulation contained the minimal concentration of propylene glycol required to bring the corticosteroid into solution. Each drug, because of its different solubility characteristics required individual formulation at each concentration.

Ostrenga *et al.* (1971b) carried out similar studies using isopropyl myristate but extended the investigation to include *in vitro* diffusion experiments with human abdominal skin and *in vivo* vasoconstriction assays. They found striking correlation between the *in vitro* release rates and the *in vivo* results. However, aqueous gels containing low concentrations of propylene glycol performed as well as the preparation containing just sufficient to solubilize the corticosteroid completely. In this case the favourable partition coefficient of the fluocinolone acetonide between the skin and the vehicle offset the decrease in concentration of the steroid in solution.

On the basis of these results, Ostrenga and his colleagues suggested the following criteria for an efficacious topical gel formulation:

(a) The concentration of diffusible drug in the vehicle should be optimized by ensuring that all the drug is in solution.
(b) The minimum amount of solvent should be used to dissolve the drug completely and yet maintain a favourable partition coefficient with the skin.
(c) The vehicle components should affect the permeability of the stratum corneum in a favourable manner.

A new type of formulation for the presentation of topical corticosteroids has been developed by Ostrenga *et al.* (1971a). This consists of a continuous solubilizing phase distributed in a solid phase of fatty alcohol and high molecular weight polyethylene glycols. FAPG is one such vehicle in which the solubilizing phase is propylene glycol which is distributed in a mixture of stearyl alcohol and a high molecular weight polyethylene glycol. *In vitro* studies showed good release rates from such formulations and *in vivo* vasoconstriction response for both open and a occluded application correlated with the percentage of drug solubilized in the vehicle. Physically the preparation combined the properties of a gel with the cream-like consistency and appearance of an emulsion system. Coldman *et al.* (1971b) showed that fluocinonide was highly effective in FAPG formulation and surpassed the high responses obtained with betamethasone valerate cream and ointment in the blanching test. Rosenberg (1971), in a double blind paired comparison in 24 patients found fluocinonide 0·05% in FAPG base to be more effective clinically in the majority of cases than betamethasone valerate cream 0·1%. However, this result could be due to the greater activity of the corticosteroid than the effects of the vehicle in which it is formulated.

The rate of diffusion of a medicament in the vehicle may also influence its rate of penetration and duration of action (Fountain *et al.*, 1969). By reducing molecular movement in the vehicle, a lowered diffusivity reduces the number of drug molecules making impact with unit area of skin surface in unit time. This may combine with an adverse partition coefficient to reduce the activity of the drug. Hadgraft *et al.* (1972) have shown that the rate of penetration of methyl nicotinate from aqueous solutions is reduced by the addition of glycerol which reduces the diffusivity of methyl nicotinate and also alters the partition coefficient in favour of the aqueous phase.

Conclusion

There is little doubt that the release of a drug from a topical preparation can be materially affected by the vehicle in which it is applied. However, the intrinsic activity of the compound itself remains the most important factor in determining its therapeutic usefulness. The marginal increases in activity obtained by pharmaceutical formulation, whilst not being paramount, are nevertheless important. Correct formulation of a topical agent will ensure that it exerts its maximal activity on the skin whilst incorrect formulation may reduce its activity. For this reason, it is inadvisable for the proprietary products of the pharmaceutical industry to be diluted with other vehicles which, whilst producing a preparation which appears to be physically acceptable, may nevertheless have interfered seriously with the release and activity of the main ingredient.

Vehicle effects should also be borne in mind when a topical agent is assessed clinically since choice of the wrong solvent or base may result in poor release of the drug and adversely effect its performance.

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Br. J. Derm. (1972), **87**, 390.

Obituary

ANDREW McCONNELL DAVIDSON

Dr Andrew Davidson, who was born in Edinburgh on November 16th, 1885, died in Vancouver on April 18th, 1972. He graduated in Edinburgh, and at the age of 26 settled in general practice in Winnipeg. He soon returned to Europe for postgraduate studies in dermatology in Edinburgh, London, Paris and Vienna. By 1913 he was back in Winnipeg in practice as a dermatologist and was soon appointed to the staff of the Winnipeg General Hospital, where he became head of the department of dermatology. Although his parents were Quakers he joined the R.A.M.C. in 1915 and was soon serving in France. In 1917 he transferred to the R.C.A.M.C., in which he attained the rank of Lieutenant-Colonel.

After returning to civilian practice in Winnipeg, Dr Davidson built up a most successful department. His research, mainly in mycology, was supported by grants from the National Research Council. He also established the first Clinic for Venereal Diseases in Winnipeg. He was a founder member of the Canadian Dermatological Association and a member of the American Dermatological Association. In 1927 he was elected an Overseas Member of the British Association of Dermatology.

In 1939 he enlisted once more in the R.C.A.M.C. and played a large part in developing improved criteria for the assessment of physical fitness. After the war he practised in Vancouver until 1967.

Dr Davidson's interests were wide. He was an enthusiastic ornithologist and a founder member of the Natural History Society of Manitoba.

Br. J. Derm. (1972) **87**, 391.

Books

Dermatology Specialty Board Review. (Multiple Choice Questions and Referenced Answers) V. J. SELMANOWITZ and N. ORENTREICH (1972). New York: Medical Examination Publishing Co. London: H. K. Lewis. £4.60.

No doubt there must be many aspiring American dermatologists to whom this book will be valuable. It contains in 18 sections 1290 multiple choice questions and the answers are almost always accurate. The questions are well-phrased, lack ambiguity and a large proportion can be answered after reading dermatology journals of the last 3 years. Antiquarian dermatology is catered for by a pre-occupation with eponyms. Rare conditions are the subject of many questions. Otherwise diagnosis and treatment are little mentioned. One is left with the impression that for examination purposes protothecosis is more important than psoriasis.

A. L. MACMILLAN

Radium Recipes for Cutaneous Cancer (The Manchester Method). J. B. HOWELL, W. J. MEREDITH, G. SWINDELL (1972). Springfield : Thomas. pp. 151. 40 figs and 23 recipes. Price £6.75

The first half of the book is devoted to the physics of radioactivity and includes the construction, care and custody of radium sources, and gives a clear exposition of the radium dosage system. The six brief chapters on clinical application of interstitial radium needle therapy for cutaneous cancer lack authority; two chapters devoted to irradiation of the ear and of the nose (rightly) stress the undesirable side-effects of this modality and makes the reader wonder why these areas were not listed as unsuitable for this form of therapy.

The final part of the monograph gives 23 plans for a variety of implants; whilst these may be useful to practitioners who only occasionally use this form of treatment, they are superfluous for radiotherapists who are conversant with the radium dosage system.

In the Introduction, the hope is expressed that the book " will prove valuable and helpful to the radiation therapist, the surgeon, the dermatologist, the oncologist and especially to those in training for these specialities who seek to master this useful treatment for cutaneous cancers ".

British dermatologists do not normally undertake the radiotherapy of skin malignancies. For those who do there are better and more detailed textbooks on radiotherapy.

F. M. BENTON
D. I. MCCALLUM

Br. J. Derm. (1972) **87**, 392.

Correspondence

Photocontact Dermatitis in a Dye Manufacturing Process

SIR,—The recent article by Gardiner *et al.* (1972) nicely demonstrates how to go about the systematic evaluation of the pathogenesis of an exogenous photodermatitis.

In describing photosensitivity states, it is desirable for authors to classify a light reaction as belonging either to the phototoxicity group or the photoallergy group, since this gives the reader a clearer picture of the underlying process. In the discussion on the paper, the above-mentioned authors did not specifically mention whether they felt the photosensitivity reaction to be phototoxic in nature. Presumably, photocontact dermatitis to Disperse Blue 35 is due to a phototoxic reaction, since the light-induced dermatosis was reproduced in those individuals allegedly not previously exposed to the chemical, its precursors or its chromatographically-separated components. Seemingly, photoallergy was not the cause since the photoallergic reaction requires previous contact with the chemical and an incubation (" sensitization ") period.

A photoallergic process due to photochemical degradation of the complete dye could be tested by pre-irradiating the complete dye for increasing time periods *in vitro*, then applying such pre-irradiated solutions to the backs of sensitive patients without further irradiation, as closed patch tests. Should one of the photo-breakdown products be allergenic, a positive patch test result would be obtained. The allergenic component could then be isolated by chromatographic studies.

In reporting the investigation of agents suspected of causing photodermatoses, it would be helpful if authors would include information on whether the compound under study fluoresces under u.v. irradiation since, in general, photoreactive compounds do show phosphorescence or fluorescence. In such cases, binding of the suspect agent to the skin can easily be demonstrated by applying the chemical directly to cryostat sections of skin and then examining these under an ultraviolet microscope (Herman and Sams, 1972).

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Retinoic Acid in the Treatment of Psoriasis

SIR,—I am interested in the latest report on the effect of Vitamin A on the psoriatic skin (Macdonald and Fry, 1972). The clinical trial follows naturally after their interesting studies (Fry *et al.*, 1970) on the histological effects. Both papers confirm Jarrett's earlier conclusions.

In referring to our closed sequential clinical trial (Whittle *et al.*, 1961) using Armitage's model (1954, 1957), which showed statistically the superiority of Vitamin A-plus-triamcinolone to triamcinolone, applied topically, $P < 0{\cdot}05$ (not "$P > 0{\cdot}05$"—a misprint!), the authors were surprised that such a result obtained using triamcinolone and retinol (Vitamin A) was not more energetically followed up ". There were several reasons for *our* not doing so.

1. *Disparity of results amongst patients.* We noted, as they did, a similar disparity. For instance, in 3 of our patients the lesions cleared completely on the vitamin treated side only: some improved dramatically in 2 weeks, while in others improvement was slow and minimal. One patient reacted adversely to both applications with eczematous dermatitis, but the vehicle containing triamcinolone gave negative patch tests. By then it became obvious that the somewhat unpredictable results detracted from its value.

2. *Relapse after treatment.* This occurred rather frequently and was also unpredictable.

3. *Superiority of other agents for routine treatment.* At the B.A.D. meeting in which our paper was read it so happened that the report on the Leeds treatment was made public (Maclennan and Hellier, 1961). Using tar baths, dithranol paste and u.v. light, their modification of the well-known Goeckerman technique, they succeeded in over 2,000 patients in getting 95% complete clearance of lesions in a median time of 18·5 days. This was probably an all-time high!

Despite these set-backs we decided to submit our paper for publication. It showed at least the favourable influence of retinol on psoriatic lesions, and confirmed Jarrett's hypothesis (Jarrett, 1958, 1960; Jarrett *et al.*, 1956, 1959a, b, 1961).

May I at this point renew my plea (Whittle, 1962) to authors to publish the interval before relapse, thus helping to evaluate success of treatment.

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Society Proceedings

ROYAL SOCIETY OF MEDICINE

SECTION OF DERMATOLOGY

President—Dr H. R. Vickers

Thursday, January 20th 1972

Malignant Atrophic Papulosis Degos' Syndrome)—Dr N. E. Jensen (for Dr R. E. Church)

A woman aged 51.

History.—Over a period of 1 year she has developed symptomless lesions scattered over the body. Previous health good except for an episode of unexplained iritis 10 years earlier and bronchitis. No abdominal symptoms. Drugs—methaqualone.

Family history.—Mother and 2 brothers diabetic. Sister has rheumatoid arthritis.

On examination.—Characteristic pink papules scattered on trunk and proximal limbs, up to 0·5 cm diameter. Early lesions pink, developing central umbilication and finally white central scar with telangiectatic border. No ocular lesions; physical examination otherwise normal.

Progress.—During the period of investigation she has had numerous complaints, including weakness, diplopia and scattered pains. These seemed related to anxiety and have improved. She is now well and continues to develop occasional skin lesions.

Investigations.—Hb. 91%, W.B.C. 10,000 per mm^3, E.S.R. 25 mm/hr. Plasma proteins, including electrophoresis, chest x-ray and barium meal, normal. No cryoglobulins. Faeces for occult blood negative.

Serum Immunoglobulins

	July 1971	*Sept.*	*Dec.*	*Normal*
IgG	910	800	810	(500–1600)
IgA	498	390	464	(125–425)
IgM	70	35	59	(47–170)

Immunofluorescent examination of skin biopsy (Dr A. M. Ward); IgA is present, with small amounts of fibrinogen, in the dermis in relation to an area of infarction. There is no immunoglobulin in the epidermis. Cells containing IgA are present in the perivascular infiltrate. No IgG, IgM, or C'3 present in the biopsy.

Histology.—(Early lesion on arm). There is an area of irregular collagen underlying a thinned epidermis. An intense chronic inflammatory infiltrate consisting of lymphocytes and macrophages is seen around small vessels and sweat ducts. Serial sections do not reveal an occluded vessel, but large quantities of mucin are present in the mid-dermis. (Dr A. Kennedy).

Comment.—The clinical appearance and histology were typical of malignant atrophic papulosis. A total of 45 cases have now been reported (Lomholt *et al.*, 1968; Mauss *et al.*, 1969) in the ratio of 3 males to 1 female. At the time of publication, 22 had bowel involvement (fatal, 20) and 6 C.N.S. involvement (fatal, 3). Twenty-two were still alive, all but 7 of these having had no evidence of systemic involvement. The interval between the onset and death was most frequently less than a year and all but 4 of the published fatalities occurred in this time. Of the survivors, 8 had had their skin lesions for 3 years or more, only 4 for over 5 years and the longest survivor is still living after 15 years. This patient was originally shown to this section in 1957 when aged 14, and is still in good health. The oldest patient is a male who has had skin lesions without complications for 3 years (Vickers, 1972). The prognosis appears better in females, as at the time of publication 2/3 of the males had died compared to 1/4 of the females.

Three cases of atrophic papulosis have been reported from America in which an increase in the serum IgA immunoglobulin fraction was described. (Roenigk and Farmer, 1968), and a similar observation has been made by Vickers (1972). It was therefore interesting to find that our patient twice showed an elevated and once a high-normal level over a period of 6 months. Immunofluorescent staining of a biopsy specimen demonstrated IgA locally in the dermis, and also within mononuclear cells. An elevated serum level is not uncommon in various skin diseases (Fraser *et al.*, 1969) and the influence of silent respiratory infections in a single case difficult to exclude, but its presence within the skin lesion seems unusual. IgG and IgM were normal in the serum and absent in the biopsy, but small amounts of fibrinogen were present in relation to the area of infarction. Patchy perivascular fibrinogen deposits were also described in one of Roenigk's cases (Roenigk and Farmer, 1968) and 4 other published cases have been investigated by a similar technique, and fibrin but no immunoglobulins detected locally (Hall-Smith, 1969; Schroeter *et al.*, 1971). Black (1971) reported the case of a man aged 28 in which fibrinolytic activity was impaired in both early and late lesions.

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Hydroa Vacciniforme.—Dr C. Ramsay (for Dr P. Hall-Smith)

A girl aged 10 years.

History.—Since the age of 5 years has had papules and blisters on exposed areas. Blisters contain yellow fluid, they crust and eventually heal to leave depressed scars. The face is usually markedly involved but lesions also develop on dorsal aspect of hands and lower forearms and on front of thighs. Condition always active in the summer; occasional lesions only at other times of the year. Has tried various sunscreens without much success.

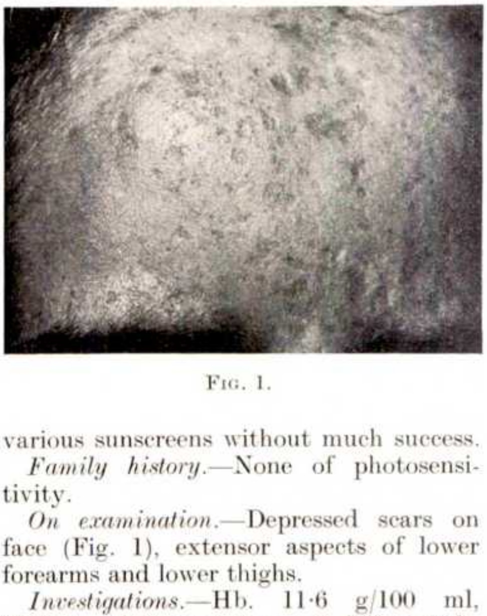

Fig. 1.

Family history.—None of photosensitivity.

On examination.—Depressed scars on face (Fig. 1), extensor aspects of lower forearms and lower thighs.

Investigations.—Hb. 11·6 g/100 ml, W.B.C. 6,200/mm^3 (normal differential). Histological Report on skin from forehead: "Apart from some loss of superficial elastica, no obvious pathology was apparent". Porphyrin screening tests negative on blood, urine and faeces. Quantitative R.B.C. porphyrins: Coproporphyrin = 2·5 μg/100 ml cells. Protoporphyrin = 12·8 μg/100 ml cells (both normal). Urinary amino-acid excretion —normal. Irradiation monochromator tests: M.E.D. at 300 nm = 52 mW.sec/cm^2 (normal) but a papule developed at this dose which is an abnormal reaction. Tests at all other wavelengths were normal.

Comment.—Hydroa vacciniforme is a very uncommon photodermatosis and this child demonstrates all of the features of the disease with a history of papules, vesicles and pustules on exposed sites which heal to leave depressed scars. The condition was first described in detail by Bazin (1862) but many of the published reports since then have confused hydroa vacciniforme with porphyria of one type or another. In the past 10 years, since erythropoietic protoporphyria has been firmly established (Magnus *et al.*, 1961), it has usually been possible to distinguish this disease from hydroa vacciniforme. The chronic stages of each of these diseases may be similar clinically with the depressed scars on exposed sites but actual blistering, one of the hallmarks of hydroa vacciniforme is uncommon in erythropoietic protoporphyria. Information on the action spectrum in hydroa vacciniforme is very scanty. Some authors have succeeded in reproducing the lesions with u.v. radiation (Schiff and Jillson, 1960) but there are no detailed reports of monochromator studies. By the use of a filter Schiff and Jillson (1960) showed that the abnormal reaction from carbon arc irradiation was due to wavelengths below 320 nm. Our monochromator tests showed that this patient developed an abnormal reaction with 300 nm irradiation, i.e. in the " sunburn " part of the spectrum.

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The following case will be published in *Proc. R. Soc. Med.*

Single Component Cryoglobulinaemic Vasculitis.—Dr J. J. Cream (for Dr F. R. Bettley and Dr C. Ramsay).

SECTION OF DERMATOLOGY

President—Dr H. R. Vickers

Thursday, February 17th, 1972

Actinic Reticuloid and " Delayed Dermographism ".—Dr C. Ramsay and Dr O. L. S. Scott.

A man aged 55.

History.—Red plaques appeared on the face in 1951. A diagnosis of Jessner's lymphocytic infiltration was made in 1958 which was supported by histology and which responded well to treatment with antimalarials. Occasional papular lesions on the face since that time. In May 1971 he worked in the sun for a short period on one day. That evening he developed erythema, oedema and oozing of the skin of exposed parts and was generally unwell. Improved with treatment but exposed areas then developed thickening and redness of the skin which has persisted. Recent spread to trunk and lower limbs.

On examination.—Erythematous scaly and papular eruption of exposed parts of head, neck and hands. Gross lichenification in some areas. Sheets of discrete papules on trunk and thighs. Otherwise normal except for scars from war wounds.

Investigations.—Hb. 15·2 g/100 ml, W.B.C. 7,000 mm^3. Normal differential. E.S.R. 17 mm/hr. Screening tests for porphyrins: blood, urine and stool negative.

Histology.—Focal spongiosis and parakeratosis with irregular acanthosis. Oedema of papillary dermis with a moderately dense infiltrate around blood vessels.

Photopatch tests.—Negative.

Irradiation monochromator tests.—An abnormal papular reaction developed at all wavelengths tested between 300 nm and 380 nm. Erythematous reaction only at 400 nm and 500 nm. Tests negative at 600 nm and 700 nm.

Tests with white fluorescent light tubes.—A bank of four 20 W tubes at a distance of 30 cm from the skin gave a papular erythematous reaction several hours after an exposure of 30 min. By using a series of plate glass filters which cut off at different points in the ultraviolet (u.v.) it was shown that the positive reaction was due to the very small amount of " sunburn " u.v. (290–320 nm) emitted by these tubes.

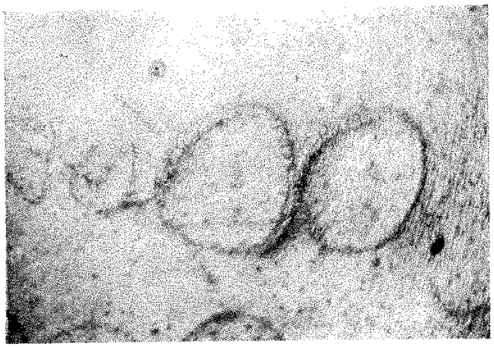

FIG. 1.—Skin of upper trunk 24 hours after trauma.

"*Delayed dermographism* ".—During investigation it was found that he developed a delayed reaction to trauma of the skin. After a latent period of several hours during which time the skin was completely normal the traumatized area became oedematous (Fig. 1) and this persisted for about 48 hours before slowly subsiding. It was thought that this represented delayed dermographism but biopsy from one of these oedematous areas showed an acute eczematous reaction. Tests for other physical urticarias were negative.

Comment.—The clinical picture, histology and irradiation monochromater tests in this patient were quite typical of actinic reticuloid (Ive *et al.*, 1969). Reaction to irradiation of the skin with white fluorescent lighting tubes occurs in a number of these patients (Brown *et al.*, 1969) but it has usually been assumed that the positive reaction is due to the long wave u.v. or visible radiation. Tests with filters in this patient showed quite definitely that his positive reaction was due to the very small amount of " sunburn " u.v. (290–320 nm) not to the long wave u.v. or visible light.

The delayed response to trauma seen in this patient was unusual and it was thought that this was an example of delayed dermographism. This condition is rare but it has been well described by Kalz *et al.* (1950) and Baughman and Jillson (1963). No histological details are given in these reports so that it is not possible to know whether the histology of delayed dermographism is identical with ordinary dermographism. However Kalz *et al.* (1950) describe and show a photograph of vesicles at the site of trauma in their case. The biopsy in the present case showed an acute eczema and it is quite possible that this reaction to trauma represents the Koebner phenomenon although it was thought that the time between trauma and the appearance of the lesion, only a few hours, was rather short.

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Actinic Reticuloid.—Dr D. S. WILKINSON.

A bricklayer, aged 42.

History.—In 1960 he developed a photodermatitis due to T_4CS. He remained only mildly affected until 1965 when a severe relapse occurred (? development of chrome sensitivity). Thereafter he became a persistent light reactor. The palms and fingers were progressively implicated, presumably due to his desultory attempts to work outdoors at bricklaying. In 1969 the exposed areas of face, neck and hands developed a pebbly lichenification. Recent sudden exacerbations due to sensitivity to PABA in sunscreen and, later, to mexenone.

On examination.—Severphe otodermatitis with marked pebbly lichenification on face, neck, ears, dorsa of hands. Chronic eczema palms and fingers.

Investigations.—All laboratory tests normal; no porphyrins.

Patch tests.—1961: T_4CS pos.: pot. dichrom. neg. 1965: T_4CS, T_3BS pos.: bithionol weak pos. 1967: pot. dichrom. 0·5%, pos. 1971: (Dr E. Cronin). T_4CS pos. (with and without light): PABA pos.: bithionol strong pos.: hexachlorophene, TCC,; T_3BS, chlorpromazine, Fentichlor pos. or weak pos. 1972: Olexenone, 1% strong pos.

Histology.—5/1971 (Dr H. J. Harris). A fairly dense infiltration by lymphocytes, plasma cells and eosinophils. There are a few giant cells. The appearances are those of actinic reticuloid. 12/1971 (Dr E. Wilson Jones). A heavy polymorphous infiltrate compatible with "actinic reticuloid".

Monochromator studies.—(Dr I. A. Magnus and Dr A. Kobza, Institute of Dermatology). 1966 Abnormal reactions to 11 u.v. bands from 300 nm–700 nm and in 1971 to fluorescent light.

Comment.—This case corresponds to the description of actinic recticuloid as given by Ive *et al.* (1969) except for his younger age. Neither of their cases, nor any of the 9 described by Heid *et al.* (1970) had originally been sensitized by halogenated salicylanilides. This case is typically resistant to treatment and his chrome sensitivity adds a further limitation to his working ability. The number of his related and unrelated sensitivity reactions raises the question of whether there is a fundamental abnormality of the reticulo-endothelial system in these patients predisposing to such reactions. One of Heid's cases was also sensitive to chromate and one of Degos *et al.* (1970) to chromate as well as to sulphonamides, PPD and metals.

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The following cases will be published in full in *Proc. R. Soc. Med.*

"Giant" Orf Lesion on the Finger Occurring in a Patient with a Lymphoma.—Dr J. Savage and Dr M. M. Black.

Anderson-Fabry Dyslipidosis.—Dr M. L. Johnson.

JOINT MEETING OF EUROPEAN SOCIETY FOR DERMATOLOGICAL RESEARCH AND THE SOCIETY FOR INVESTIGATIVE DERMATOLOGY

Amsterdam May 17–19th, 1972

The success or failure of any scientific meeting depends on an indefinable mixture of the numbers and the enthusiasm of the participants, the quality of the presentations and the amount of useful contact made between individuals from different centres. Other factors such as the physical facilities and the social functions provided for the meeting are also important, but in themselves do not determine the outcome of a meeting.

By any standards, the first co-operative venture of the SID and the ESDR was an unqualified success. The meeting was held in 5 sessions which were spread over 3 days, during which time 48 papers were read and 8 lunch-time "workshop" discussion groups were held. The interest of the meeting was sustained by the uniformly high quality of the papers both as far as content and presentation were concerned. Speakers were concise and fluent and there was a welcome air of professionalism about all the sessions.

Many of the papers were on pharmacological topics. Of particular interest in this subject area was a paper on the "Mechanism of Depigmentation by Hydroquinone" by Jimbow, Obata and Pathak in which the cytological changes produced by the action of hydroquinone were discussed in detail. Another presentation on the pharmacological themes was one entitled "Effects of Anti-inflammatory Drugs on Prostaglandin Synthesis by the Skin" by Greaves and McDonald Gibson, in which the synthesis of prostaglandins by adult skin was demonstrated *in vitro* and the usefulness shown of this model for the study of anti-inflammatory drugs. The pathophysiology of pigmentation was also a popular theme—one of the lunch-time "workshops" was on "Melanin Pigmentation" and was chaired by Drs A. B. Lerner and H. Rorsman. Papers that were read in this subject area included one entitled "Hyperpigmentation, Melanosome Size and Distribution Patterns of Melanosomes" by Konrad and Wolff in which ultrastructural studies showed that the number, size and location of melanosomes differ in different lesions and that there is a tendency for small melanosomes to be aggregated and for larger melanosomes to occur singly.

There was an extremely useful lunch-time session on Tissue Culture, skilfully steered by Drs Funan Hu and Michel Prunieras and throughout the entire meeting there was an emphasis on *in vitro* techniques. A paper on this theme by Dahl and Shuster from Newcastle described an assay technique for "Chalone" activity in epidermal extract and another from Miami by Hsia, Wright, Mandy and Halprin reported a decrease in the level of adenylcyclase in psoriatic plaques compared to normal skin.

There were few papers concerning specific diseases although there was one entitled "Epidermal Kinetics in Lichen Planus", by Marks, Black and Wilson Jones in which the migration of keratinocytes into the lesion of lichen planus from the uninvolved margins was detected. There was a paper on "Fine Structure Studies of Families with Pseudoxanthoma Elasticum" by Ross and Fialkow and another by Pinnell and Krane concerning a new heritable disorder of connective tissue characterized by a deficiency in the hydroxylysine content of collagen.

When last year's meeting of the ESDR at Nordwijk and this year's combined meeting with the SID are considered it is evident that the ESDR fulfils an important function in bringing together for a few days all who are interested in the investigative aspects of dermatology. It is clear that those who have just started dermatological training should be encouraged to attend these meetings as they should help to induce a keen and critical attitude to the subject. It should be noted that it is intended to hold the ESDR meeting annually each April or May in Holland and that all who are interested are welcome to attend. This year's President is Professor S. Shuster of Newcastle who will be pleased to answer queries concerning the Society and its meetings.

It is hoped that succeeding annual meetings will be well supported and that this venture in European co-operation will continue to be a resounding success. Those interested in submiting a paper to be read at the next meeting of the Society should obtain a form for this purpose from the Secretary before December 15th, 1972.

The Society is anxious to encourage new members and applications should be made to the Secretary, Dr M. Prunieras, 29 rue Manin, Paris 19e, with their c.v. before December 31st, 1972.

Br. J. Derm. (1972) **87,** 400.

Quarterly Review

CONTACT DERMATITIS XIX

Sensitivity to Chromium in Cement Dermatitis

Chromate sensitivity was reported in men with cement dermatitis by Bonnevie (1939), but it was attributed to the wearing of leather gloves and its relationship to cement remained uncertain until the work of Jaeger and Pelloni (1950). They obtained positive patch tests to potassium dichromate in 30 of 32 patients with cement eczema, and established the significance of this allergy by demonstrating the presence of minute quantities of chromium in different samples of cement. The chromium in cement is derived from the raw materials of which it is composed, mainly from the clay and to a less extent from the coal ash and chalk. It is converted into chromate in the kilns, where high temperatures are used to dry the wet cement slurry (Johnston and Calnan, 1958).

The importance of chromate sensitivity in the initiation, severity and perpetuation of cement dermatitis has remained uncertain. Reports of the frequency of allergy to chromate in these patients has been variously reported as 100% (Frey, 1952) and 52% (Bett, 1958). The average incidence of all the reports is 83% (Høvding, 1970).

To elucidate the incidence and importance of sensitivity to chromate in men working with cement Høvding (1970) studied the masons and hodmen (bricklayers and bricklayers' labourers) in the city of Bergen. He chose this occupation as they form a well-defined and stable group of workers. After an initial postal questionnaire he visited the men on their working sites, examined and then patch tested each one to potassium dichromate 0·5% in water. The whole group of 366 men was composed of 221 masons and 145 hodmen. The average age of the masons was 42 years and they had done this work for an average of 22 years. The hodmen, which included more casual labour, had an average age of 49 years, and their average length of employment was 18 years.

On patch testing 11% (25/221) of the masons and 5·5% (8/145) of the hodmen reacted to chromate, giving an overall incidence of 9% with chromate sensitivity. The higher incidence in the masons was thought to be due to their greater contact with wet cement. Sensitivity was also more frequent in men over the age of 50 years and in those with prolonged exposure who had been doing the job for over 40 years.

On clinical examination 88% of the men had normal skins, 7% had some dryness and cracking of their hands from the irritant effect of cement and 6% (21/366) had eczema. The irritant changes were as common in chromate positive as in chromate negative men, whereas of those negative to chromate, only 1·2% (4/333) had eczema, while of those who were sensitive to chromate 52% (17/33) had eczema. Of the 21 men with eczema, 17 (81%) were sensitive to dichromate. Although these figures show that those with cement eczema are very likely to be sensitive to dichromate, they equally emphasize that the presence of dichromate sensitivity in a man does not necessarily cause eczema, as only 17/33 positive reactors had eczema when they were examined.

Of these 14 chromate sensitive men without eczema, 5 had a past history of occupational eczema, but 9 had no past history of eczema nor other skin complaint.

In this group the prognosis of those with eczema was not as gloomy as that usually given. Although in the majority the eczema ran a chronic course there were periods of improvement and even times when the skin returned to normal. The development of eczema was rarely incapacitating and did not necessitate a change of occupation.

This interesting and careful study gives a very good assessment of the incidence and clinical significance of chromate sensitivity in a group of men who have worked with cement for many years.

Necrotic Reactions to Applied Medicaments

Necrosis of the skin is seldom an expected result of topical therapy except where caustics are purposefully used. The reactions observed some years ago (Coles and Wilkinson, 1965) due to the application of a quaternary ammonium compound, dequalinium, when it was applied to quasi-occluded areas of the skin, were unusual and were not predicted in the light of earlier experience when the medicament was used in other sites. Prompt withdrawal of the creams and lotions concerned terminated the outbreak, though two pharmacists, who had personally used some of the retained stock, were seen a few years ago with a similar clinical picture of necrotizing balanitis. Similar necrotic lesions have also been reported from Germany (Braun-Falco and Lukacs, 1970).

Occasionally, necrotic reactions are seen from accidental or intended application of caustics and a diagnosis of dermatitis artefacta was, not unnaturally, suspected in the first few cases of penile necrosis seen. Violent, and occasionally necrotic, reactions may also occur from the mistaken application of some medicaments that are innocuous when used at a recommended strength but violently irritant when this is greatly exceeded, e.g. cetrimide, potassium permanganate (as crystals) and phenol.

Dermatologists are, therefore, alerted to the possibility that necrotic changes may occasionally follow the use of applied medicaments which are, in general experience, therapeutically helpful and harmless. It is, nevertheless, surprising that such reaction should be reported from the use of such time-honoured remedies as gentian violet and brilliant green (triphenylmethane dyes) (Björnberg and Mobacken, 1972). A warning note had been given by Jennison and Llewelyn-Jones in 1957. They treated 36 patients suffering from monilial vaginitis with 1% aqueous gentian violet. Six developed a severe local reaction. Other isolated references in the literature record necrotic inflammatory reactions, particularly from the use of 2% gentian violet after dermabrasion. Bjornberg and Mobacken described 3 patients out of several who developed necrotic lesions after the application of 1% gentian violet in aqueous solution to the skin; one involved the napkin area of an infant, one the scrotum and one the vulva. As with dequalinium, these sites seem particularly prone to necrotic reactions from medicaments. A severe irritant dermatitis on the scrotum of patients sitting on undissolved bath concentrates of hexachlorophane affords another example (Baker *et al.*, 1969). The authors were able to reproduce the necrotic lesions experimentally on stripped skin and on guinea-pigs. Though their experience has led them to be cautious in advocating the use of these medicaments, the authors have really only emphasized something which should be widely recognized. The skin is not a homogeneous organ: nature has no need of polythene to procure occlusion; a topical application tolerated in most areas may produce quite different effects when used in certain vulnerable regions.

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News and Notices

Institute of Dermatology, University of London

Applications are invited from those wishing to be considered for inclusion in the Diploma in Dermatology Course commencing October 3rd, 1973. Applications should be submitted before the end of the year, and applicants will be notified in March 1973 as to whether or not their application has been successful. Application forms may be obtained from the Dean, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London, WC2H 7BJ.

Diploma in Dermatology, Institute of Dermatology, London

The following were successful in the Diploma Examination which was held in July this year.

Dr Khalil Ebrahim Al-Arrayed, Dr Muetabar Al-Salihi, Dr Donald Nandasiri Atukorala*, Dr Mark Bevan Duffill*, Dr Onsi Samuel Ghobrial, Dr Ramesh Kumar Gulati, Dr Ahmad Samir Kayali, Dr Abdelaziz Ahmed Klander, Dr Alfred Wing Kuen Lau, Dr Sunil Harilal Maniar, Dr Mohammed Moshir, Dr Ezzeldin Hassan Mustafa, Dr Ahmad Daudpota Nisar, Dr Farangis Rastegar, Dr Mohammed Awad Rusheidat, Dr Ram Kumar Shrestha, Dr Mohammad Haram Ziaiy.

* Awarded a mark of distinction.

Scottish Dermatological Society

President: Dr W. Frain-Bell

Annual General Meeting, Aberdeen, June 2nd and 3rd, 1972

Scientific Section

The following papers were presented:

The Effect of Enzyme Detergents on Insensible Water Loss. Rowan Adams, C. L. Hales and E. J. Raffle.

Clinical and Pathological Features of Prognostic Significance in the Presentation of Malignant Melanoma. Rona M. MacKie.

Thermography in Relation to Dermatology. A. M. Murray.

The Effects of Corticosteroid Injections at Sites of Skin Damage. L. Stankler and S. W. B. Ewen.

Stripping for Candida. R. Barnetson and L. Milne.

Hypertension, Neurofibromatosis and Pregnancy. R. A. Main and G. H. Swapp.

Studies of Nalidixic Acid Phototoxicity: Photohaemolysis. B. E. Johnson.

Long Term Effects of Hydroxyurea in Psoriasis. M. G. C. Dahl and J. S. Comaish.

Allergy to Metals—*In Vitro* Experiments in Subjects with Nickel Contact Dermatitis. F. Hutchinson, E. J. Raffle and T. M. MacLeod.

Alfred Marchionini Prizes

On the occasion of the XIVth International Congress of Dermatology in Vienna the Marchionini Prizes were awarded. The Alfred Marchionini Medal in gold was conferred on Professor M. Monacelli of Rome, whilst the Alfred Marchionini Prize, worth DM 10,000 was divided between Professor R. Milbradt of Frankfurt and Dr Gebhart of Vienna.

The Council of the Alfred Marchionini Foundation, on the occasion of the Annual Meeting of the British Association of Dermatology in London on July 8th, 1972, awarded the Alfred Marchionini Medal in gold to Dr G. B. Dowling. Professor H. Götz, the Chairman of the Council, in presenting the medal in the presence of the founder Kurt Herrmann, paid tribute to Dr Dowling's services, with particular reference to his efforts to restore and foster contacts between British and German dermatologist ssince the war.

Preliminary Scientific Programme of the Third World Congress of the International Society of Tropical Dermatology

The Board of Directors in charge of preparing the scientific programme of the 3rd World Congress scheduled for September 1st to 5th, 1974, in São-Paolo met under the Chairmanship of Professor Sebastiao Sampaio on May 24th at the Palazzo del Cinema, Venice, Italy.

Each morning one of the following themes will be presented in the main Assembly Hall: (1) Geographical ecology of skin diseases, (2) Skin cancer, (3) Mycoses, superficial, deep and systemic, and (4) Nutritional and metabolic dermatoses. In the afternoons the following symposia and Round Table Conferences are planned: (1) Pemphigus group, (2) Acne and seborrhoeic diseases, (3) Hypersensitivity dermatoses, (4) Leprosy, (5) The role of genetics in dermatology, (6) Treponematoses and venereal diseases, (7) Vasculitis, (8) Filariases, (9) Pseudo-malignancies, (10) Photobiology, (11) Atypical mycobacterial dermatoses, (12) Viral dermatoses, (13) Parasitic dermatoses, (14) Improved investigative methods in dermatology, and (15) Free communications. Patient demonstrations, Clinico-pathological presentations, and Luncheon conferences are also planned.

For additional details please communicate with Professor Raymundo Castro, Secretary-Treasurer of the Congress, Hospital das Clinicas, Department of Tropical Medicine and Dermatology, Caixa Postal 8091, São-Paolo, Brasil.

Correction

Dr W. B. Shelley writes "We regret that a typographical error made by us has occasioned confusion. The caption for Fig. 7 should have read: Direct immunofluorescent staining for IgG. Intercellular immunofluorescence of lower epidermis of lesion seen in Fig. 3."

(See *Br. J. Derm.*, **86**, 122).

Psoriasis Research Award for 1972

The Department of Dermatology, Baylor College of Medicine, Houston, Texas, announces the Mr and Mrs J. N. Taub International Memorial Award for Psoriasis Research for 1972.

This annual award of One Thousand Dollars was established in 1957 by Carol and Henry J. N. Taub in honour of their grandparents, Mr and Mrs J. N. Taub. Their establishment of this programme carried with it the hope that the award would be an incentive to develop a cure to alleviate one of the problems of mankind.

The award is designed for the individual or group whose investigative work in this field had been the most outstanding. Names of applicants for the award, together with supporting data, should be submitted for consideration by December 1st. Results of the research must have been presented before a dermatological medical society or published in a recognized medical journal.

The award's recipient will be determined by a committee of seven men. If, in the committee's opinion, none of the applications reflects outstanding research, no award will be given for the year.

All communications concerning the award should be addressed to Dr John M. Knox, Professor and Chairman, Department of Dermatology, Baylor College of Medicine, Houston, Texas, 77025, U.S.A.

Appointment

Dr Rodney H. Macdonald has been appointed Consultant Dermatologist to the Roch, dale and District, North East Manchester, Booth Hall and Monsall groups of hospitals and Manchester and Salford Hospital for Skin Diseases. He graduated M.B., Ch.B., in 1963 at Edinburgh University. In 1966 he obtained the D. Obst. R.C.O.G. He held various house officer appointments in Edinburgh including one in dermatology. After a year as Senior House Officer in the Poisoning Treatment Centre of Edinburgh Royal Infirmary he obtained the M.R.C.P. (Edinburgh) in 1967. He then became Registrar in dermatology at Addenbrooke's Hospital, Cambridge, and thereafter Senior Registrar at the Western Infirmary, Glasgow.

His published work includes papers on clinical toxicology, on the distribution of skin lesions, and on the use of Strontium 90 therapy in dermatology.

Correction

In the article entitled "Chalone Control of Mitotic Activity in Eccrine Sweat Glands" by W. S. Bullough and Johanna U. R. Deol, a typing error in the manuscript was unfortunately overlooked. The figure −26% in the last line of Table III should read −4%.

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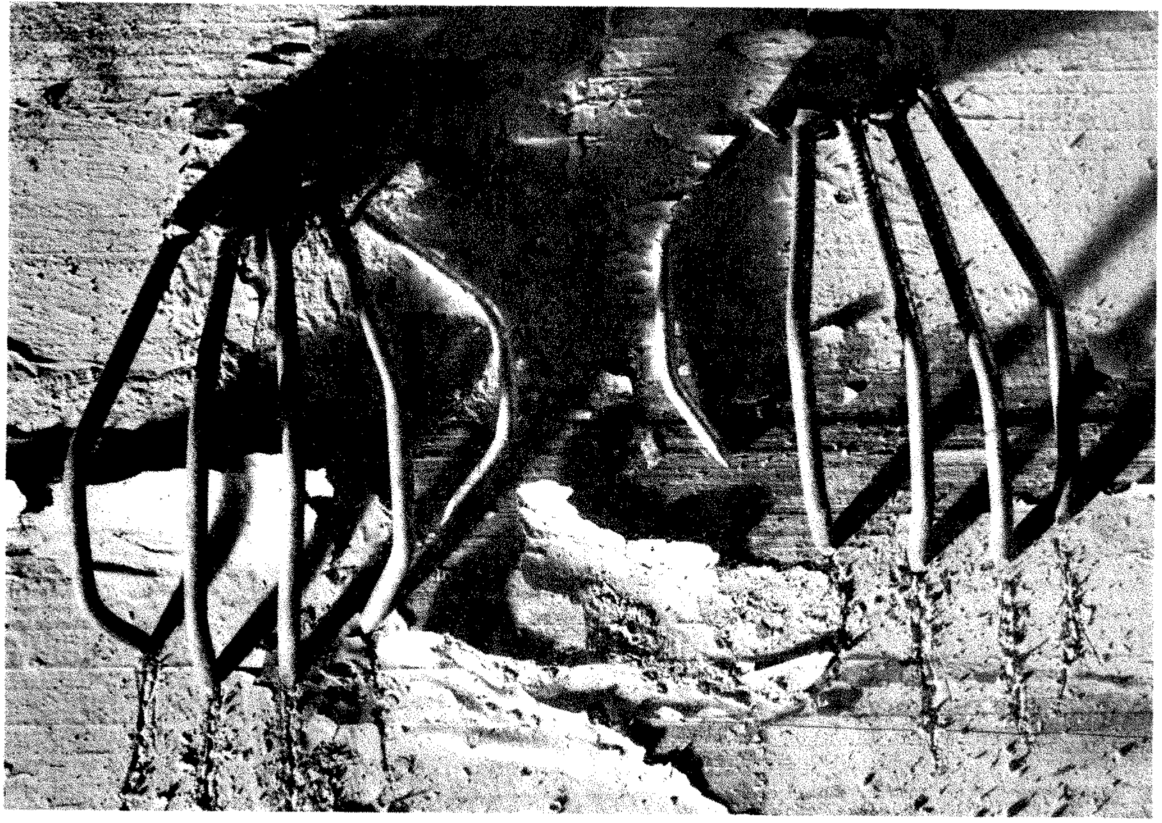

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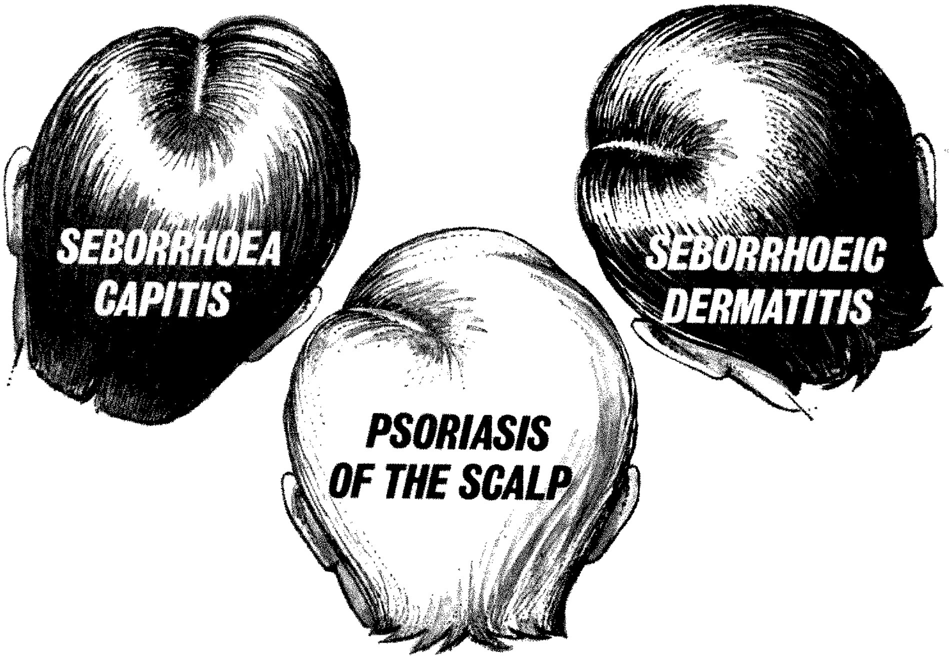

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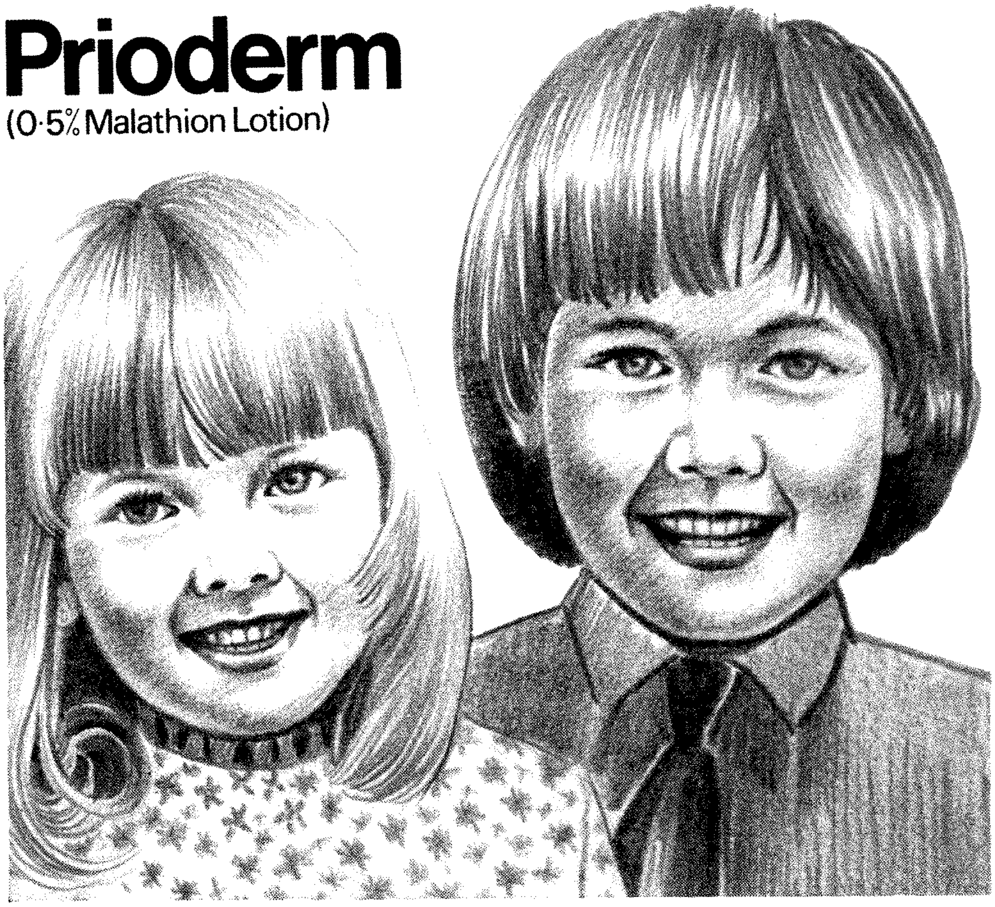

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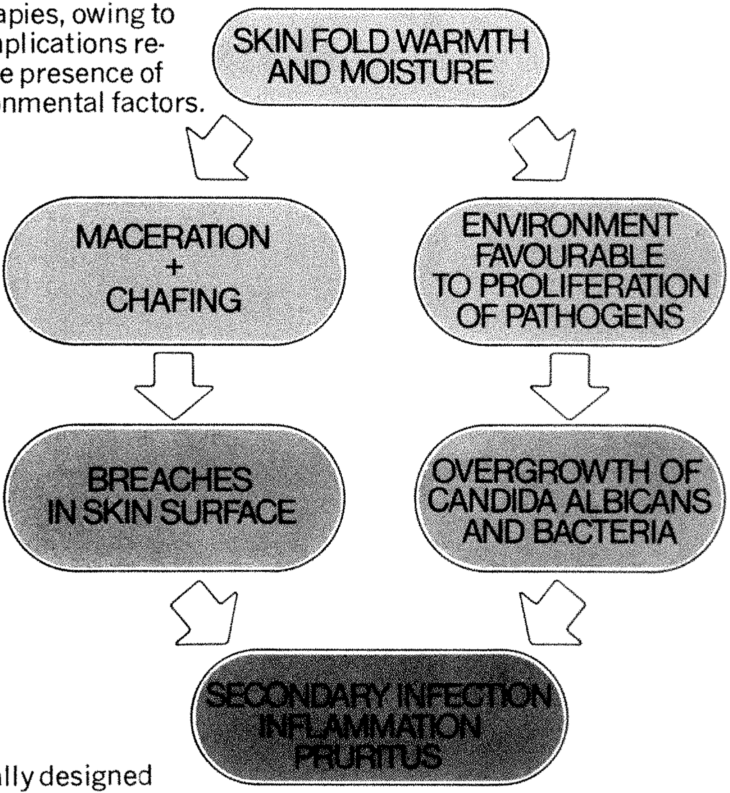

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CONTENTS (*continued*)

Comment

Recent Progress in the Formulation of Vehicles for Topical Applications
J. W. Hadgraft 386

Obituary

Andrew McConnell Davidson 390

Books

Dermatology Specialty Board Review (Selmanowitz and Orentreich); Radium Recipes for Cutaneous Cancer (The Manchester Method) (Howell, Meredith and Swindell) 391

Correspondence

Photocontact Dermatitis in a Dye Manufacturing Process
Peter S. Herman 392
Retinoic Acid in the Treatment of Psoriasis
C. Howard Whittle 392

Society Proceedings

Royal Society of Medicine, January 20th, 1972 394
Royal Society of Medicine, February 17th, 1972 396
Joint Meeting of European Society for Dermatological Research and the Society for Investigative Dermatology, May 17th–19th, 1972 . . 398

Quarterly Review

Contact Dermatitis XX. Sensitivity to Chromium in Cement Dermatitis. Necrotic Reactions to Applied Medicaments
D. S. Wilkinson and Etain Cronin 400

News and Notices 402

19 DEC 1972

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

Hereditary Inflammatory Vasculitis with Persistent Nodules. A Genetically-determined New Entity Probably Related to Lupus Erythematosus
William B. Reed, Robert F. Bergeron, Denny Tuffanelli and Edward Wilson Jones 299
Atypical Erythrokeratoderma with Deafness, Physical Retardation and Peripheral Neuropathy
J. Martin Beare, N. C. Nevin, P. Froggatt, D. C. Kernohan and Ingrid V. Allen 308
Perioral Dermatitis: Aetiology and Treatment with Tetracycline
Angus Macdonald and Michael Feiwel 315
Nickel Antibodies
Louis Forman and Suzanne Alexander 320
Pilo-sebaceous Duct Obstruction and Acne
R. L. Holmes, M. Williams and W. J. Cunliffe 327
Age and Sex Variation in Skin Surface Lipid Composition and Sebum Excretion Rate
J. A. Cotterill, W. J. Cunliffe, B. Williamson and L. Bulusu . 333
Lichen Planus; an Inborn Error of Metabolism
D. W. K. Cotton, José J. M. A. van den Hurk and W. B. J. M. van der Staak 341
The Control of Epidermal Thickness
William S. Bullough 347
Ultrastructural Study of the Cutaneous Elastic Fibres in Lupus Erythematosus
D. Schmitt, J. Thivolet and H. Perrot 355
Hair Diameter in Female Baldness
David Jackson, R. E. Church and F. J. Ebling 361
Abnormal Hair Shafts in Psoriasis on Scanning Electron Microscopy
Edward Wyatt, Eva Bottoms and Stanley Comaish . . . 368

Case Report

Post Kala-azar Dermal Leishmaniasis
D. D. Munro, Anthony du Vivier and W. H. Jopling . . . 374

Pharmacology and Treatment

Ultraviolet Therapy of Psoriasis: a Critical Study
E. Young 379
Griseofulvin Therapy in Lichen Planus. A Double-blind Controlled Trial
V. N. Sehgal, G. J. S. Abraham and Gauri Bazaz Malik . . . 383

(*Continued on* p. iii *of cover*)

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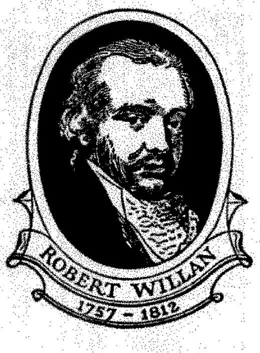

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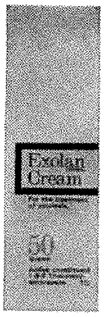

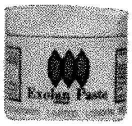

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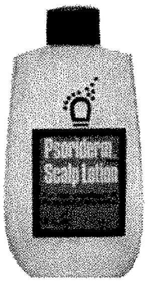

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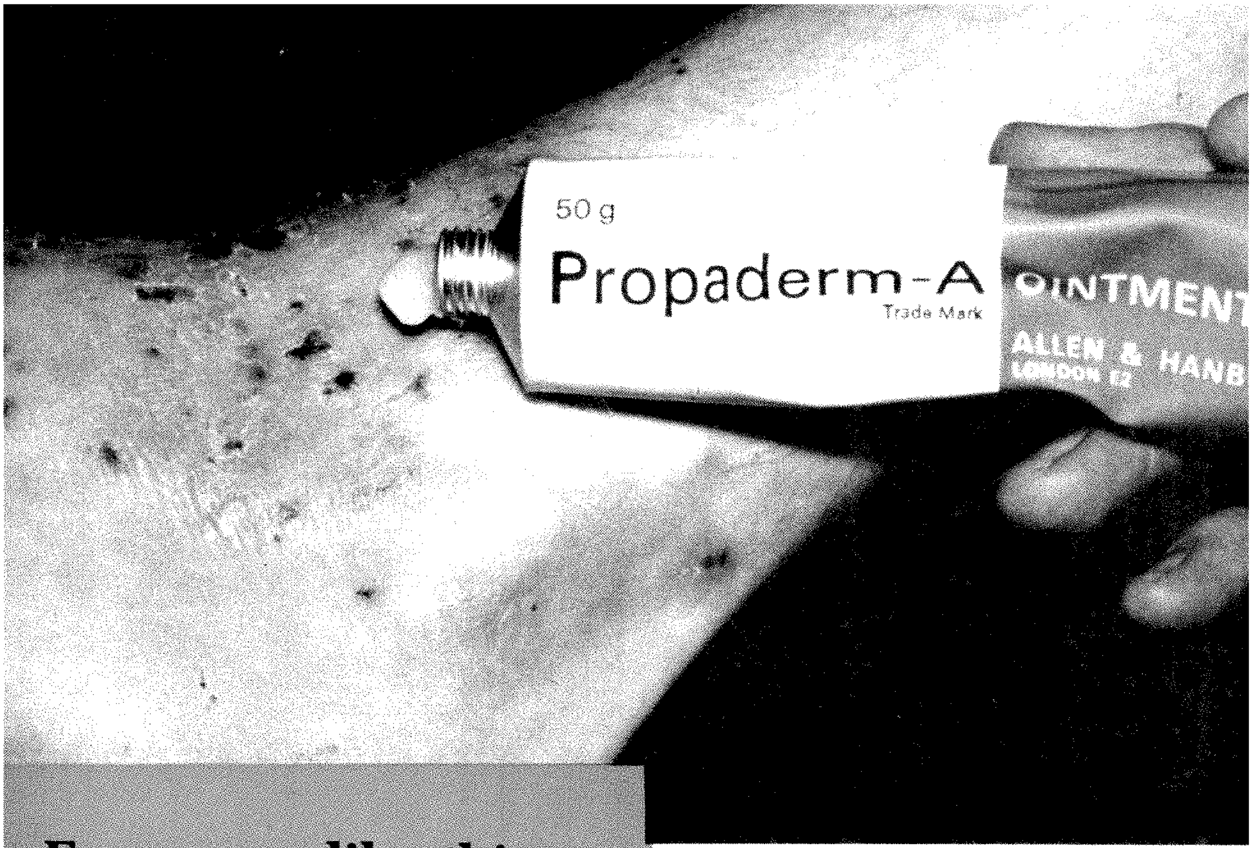

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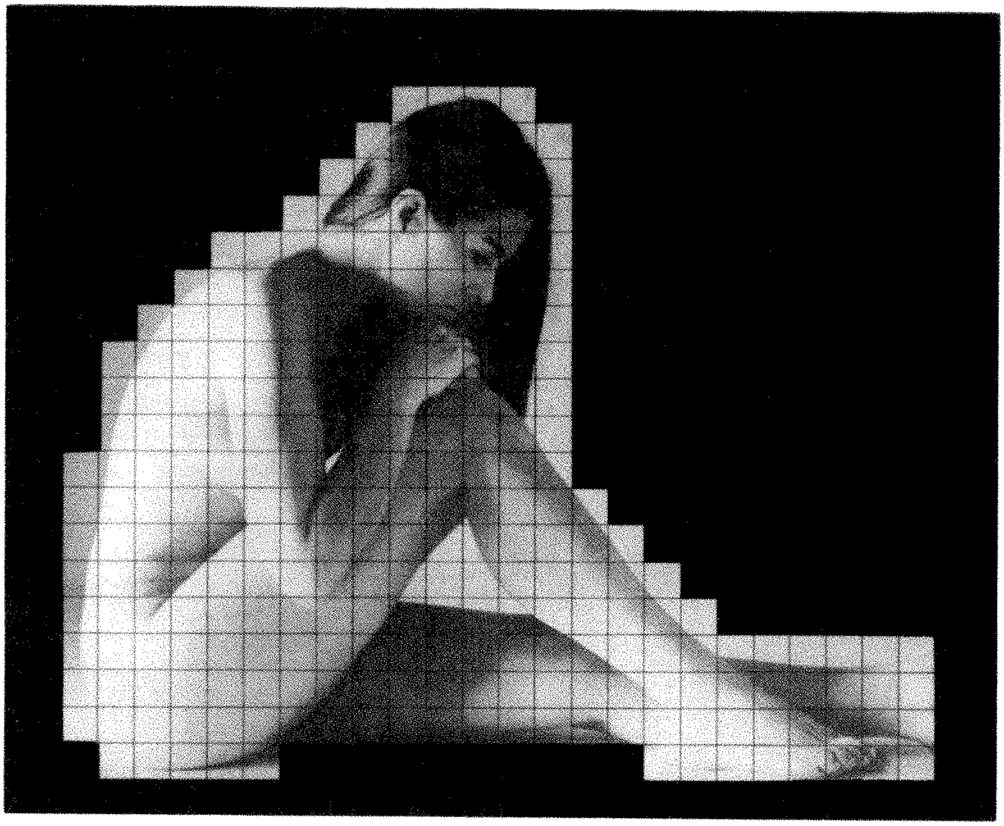

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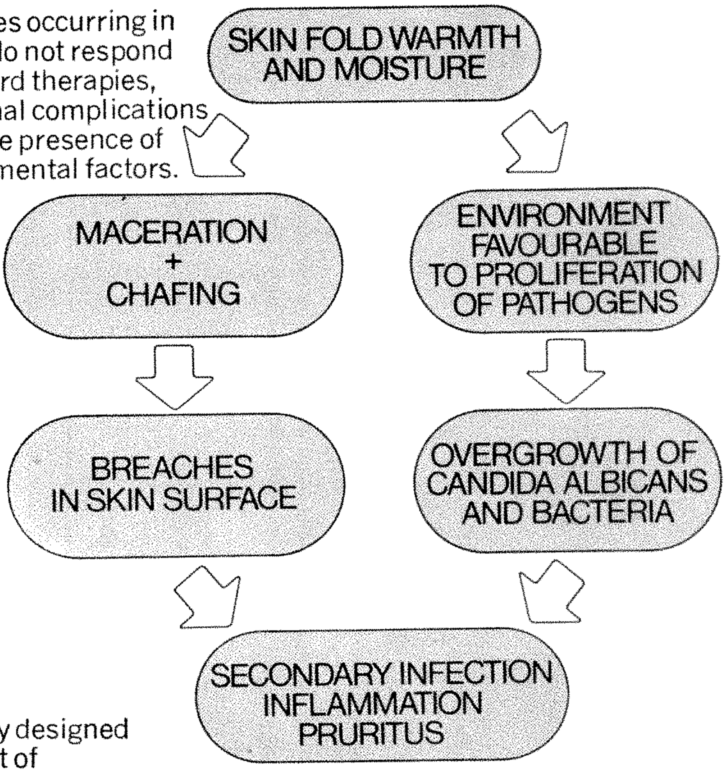
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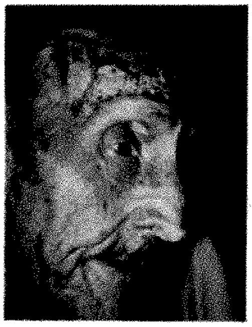

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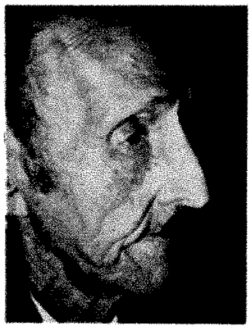

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1. Millikan L.E. and Anderson P.C., Miss.Med., 1971, *68*, 33.
2. Litwin M.S., J.La.med.Soc., 1972, *124*, 33.

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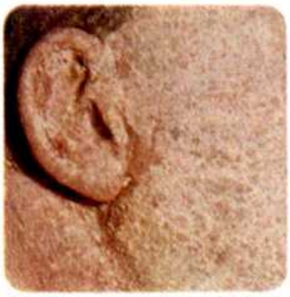
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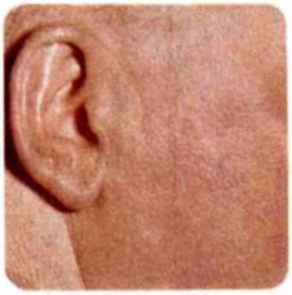
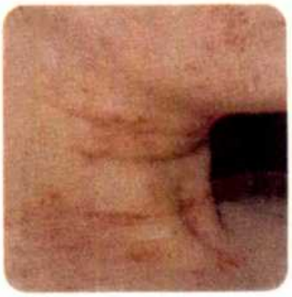
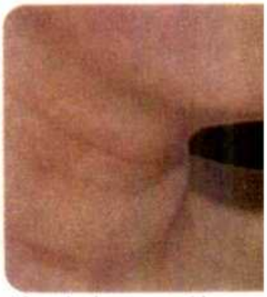
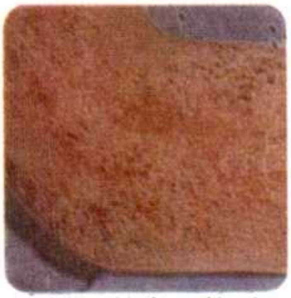
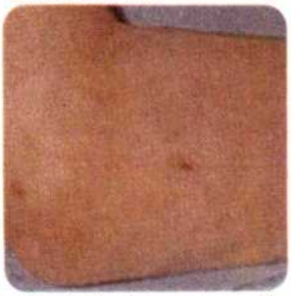

After 7 days' treatment with Betnovate Ointment


Atopic eczema


After 4 days' treatment with Betnovate Ointment


Atopic dermatitis of 3 years' duration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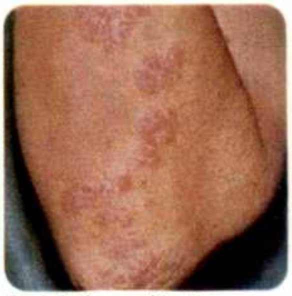
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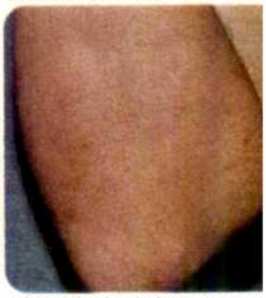
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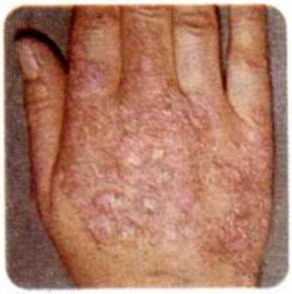
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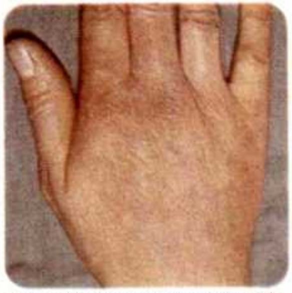
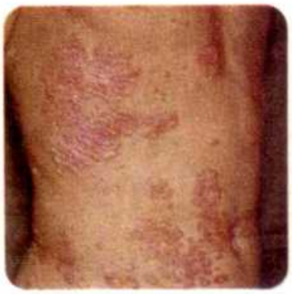

After 9 days' occlusive treatment with Betnovate Ointment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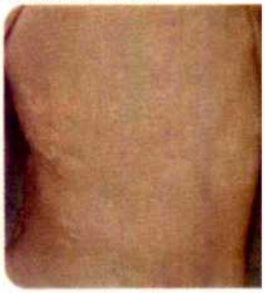
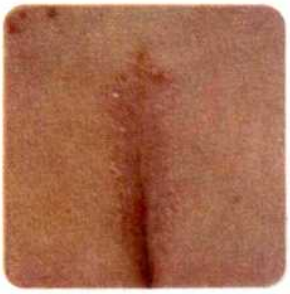
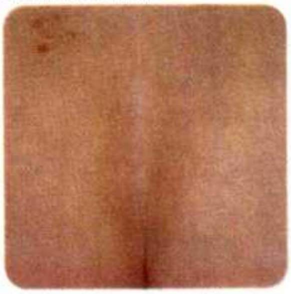
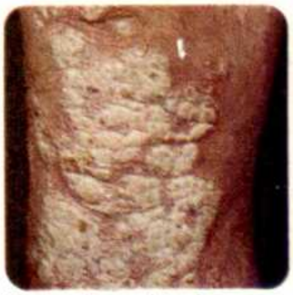
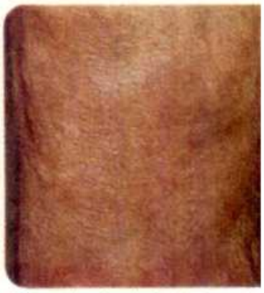

After 21 days' occlusive treatment w
Betnovate


Flexural psoriasis of 15 years' duration


After 4 weeks' treatment with


Psoriasis of 51 years' duration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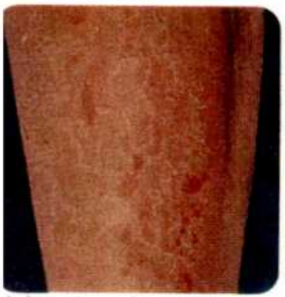
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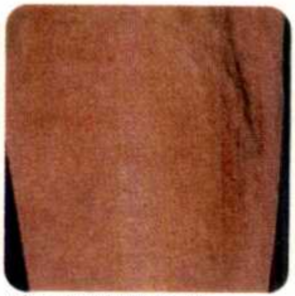
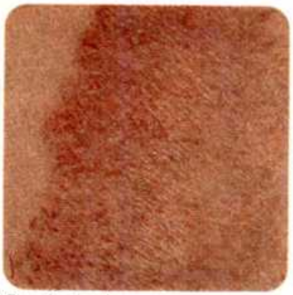

After 7 days' treatment with Betnovate Cream


Constitutional eczema

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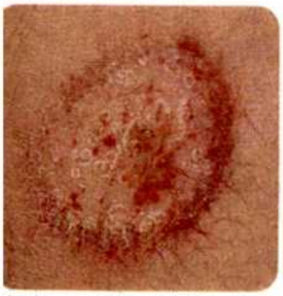
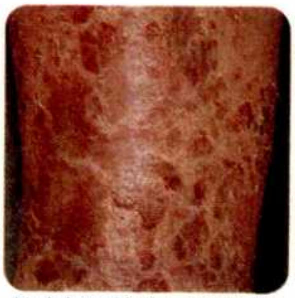
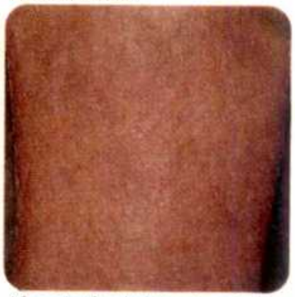

Discoid eczema of 9 months' duration

After 2 weeks' occlusive treatment with Betnovate Ointment


Atopic dermatitis of 12 years' duration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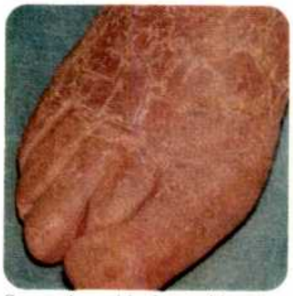
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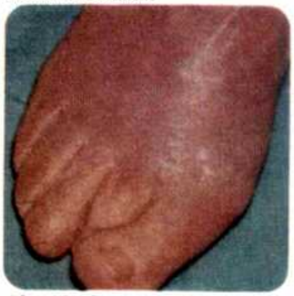
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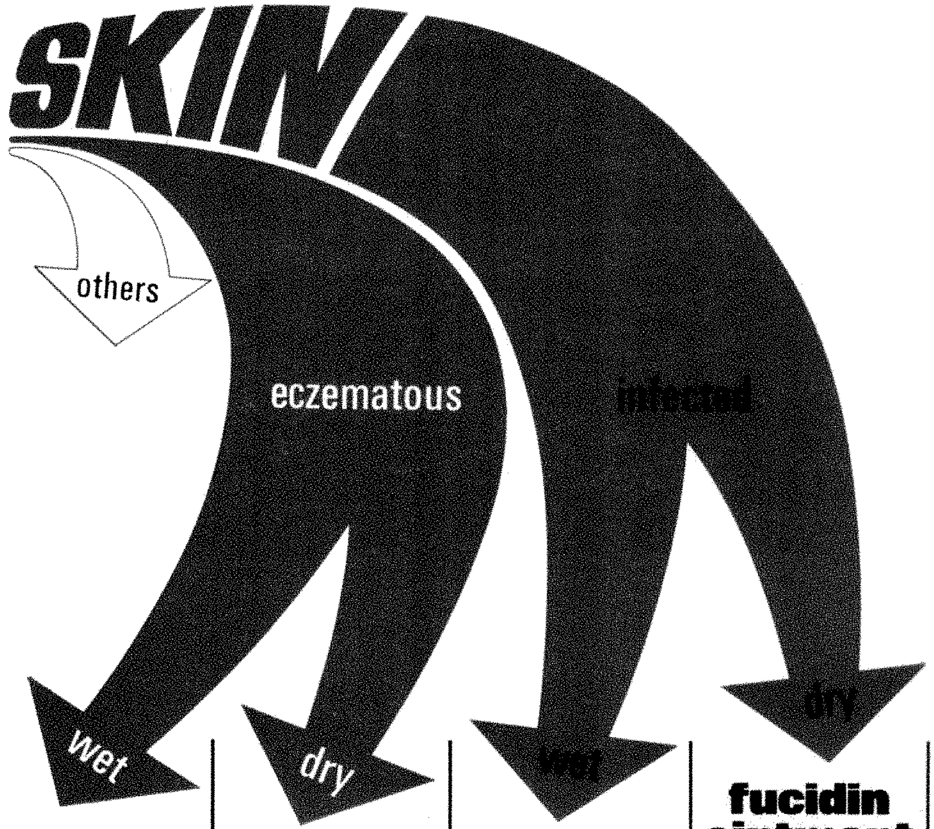

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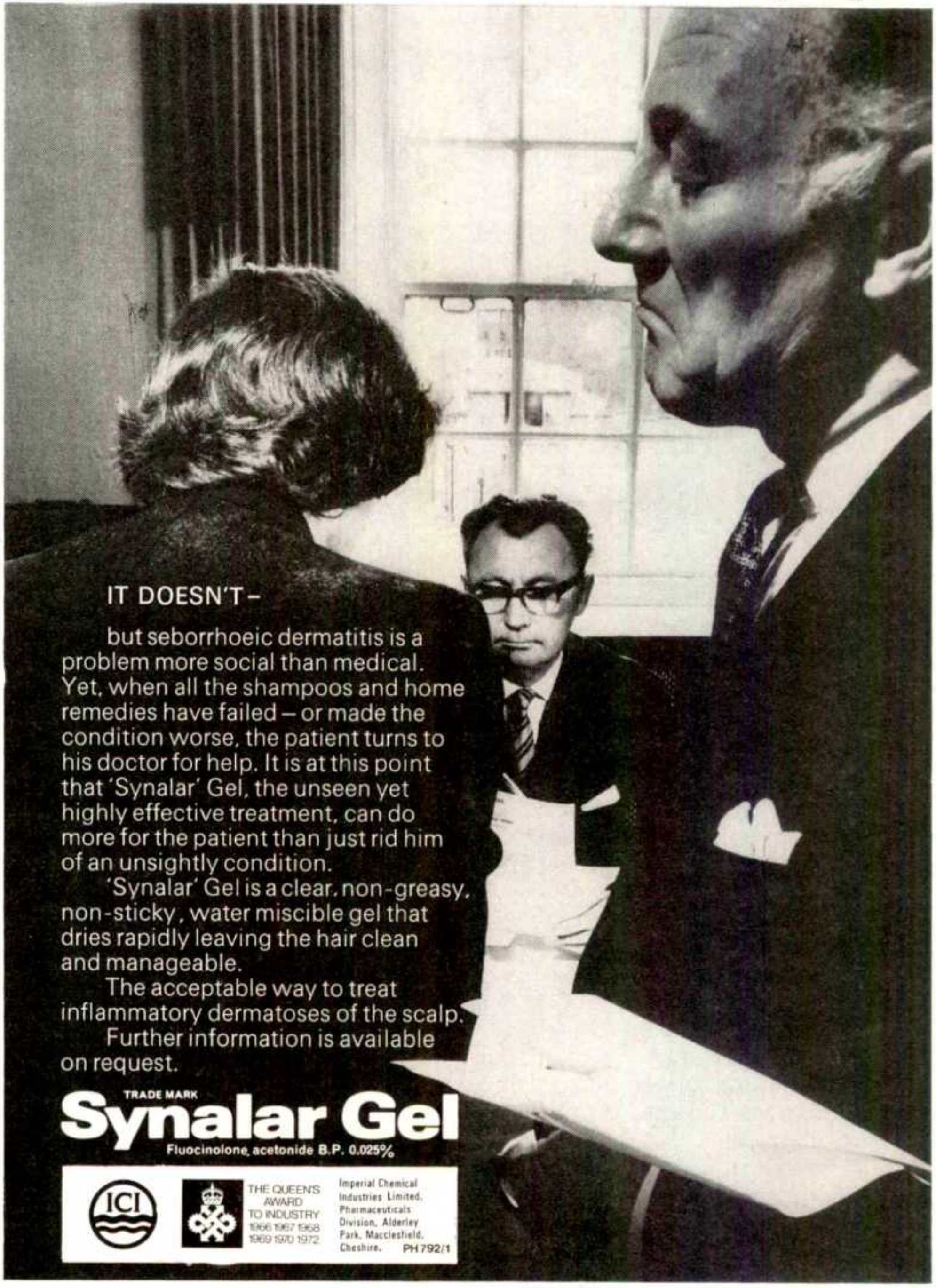

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THE NATURAL HISTORY OF PARAPSORIASIS EN PLAQUES (CHRONIC SUPERFICIAL DERMATITIS) AND PRERETICULOTIC POIKILODERMA

PETER D. SAMMAN

Summary.—The term parapsoriasis en plaques includes 2 quite unrelated conditions, one eczematous and benign, the other prereticulotic and potentially malignant. These 2 conditions are described and their features are compared in detail. In England, especially the south, the benign form is a good deal more common than the premalignant. The reason why parapsoriasis en plaques is regarded as a potentially serious disease is thus due largely to nomenclature, but also in part to the fact that, in the early stages of the classical Alibert form of mycosis fungoides and in prereticulotic poikiloderma, the appearances can closely simulate the benign type of parapsoriasis. Careful clinical observation and histological examination should, however, serve to distinguish between them.

HISTORY AND NOMENCLATURE

THE term parapsoriasis was used by Brocq (1902) to describe a group of disorders which included plaque, lichenoid and guttate forms. A number of these conditions had earlier been described under separate names. The group has since been modified and, in his classic " Fiftieth Anniversary of Parapsoriasis ", Civatte (1951) separated clearly the guttate forms, which are now more generally known as pityriasis lichenoides chronica and pityriasis lichenoides acuta, the latter also being known as Mucha–Habermann disease. This left parapsoriasis en plaques and lichenoid parapsoriasis.

Poikiloderma atrophicans vascularis was the name given by Jacobi (1908) to a condition which subsequent writers have considered to be a case of dermatomyositis (Dowling and Freudenthal, 1938). The name was taken up by Zinsser (1910), Marchionini and Besser (1932) and others to describe a superficially similar disorder which is, however, probably a rare congenital disorder characterized by skin changes, dystrophic nails and leukoplakia. This condition is thought by Cole *et al.* (1957) to be the same as dyskeratosis congenita and probably also Fanconi's anaemia. The same term, however, was used by Lane (1921) to describe a case which later developed into Hodgkin's disease (Lane, 1923), and this is usually accepted as the type case for the prereticulotic poikilodermas.

Unfortunately, the terms poikiloderma atrophicans vasculare, parapsoriasis en plaques and lichenoid parapsoriasis have at times all been used as synonyms, but they embrace 2 entirely unrelated conditions. These are considered independently in this report, one entirely benign and the other potentially malignant.

Accepted for publication May 2nd, 1972.

PARAPSORIASIS EN PLAQUES (BENIGN TYPE) OR CHRONIC SUPERFICIAL DERMATITIS

The benign form is characterized by patches of erythema, usually fairly small, round, oval or finger-like in shape, situated on trunk and limbs. They normally remain discrete. They are covered with a fine scale and have a slightly wrinkled surface. On the lower limbs, in particular, larger patches may be found. In any one patient the colour of the plaques is uniform, but it varies from patient to patient. The plaques are remarkably stubborn, responding to treatment with steroid creams or to natural and artificial sunlight, but usually reappearing promptly when treatment is discontinued. The patches increase in number for a time and then remain relatively constant for several years. A small minority of cases clears entirely. There may be slight irritation, but this is minimal, and most patients have no complaints except for the appearance. The histological changes are also minimal and may be interpreted as a very mild eczema.

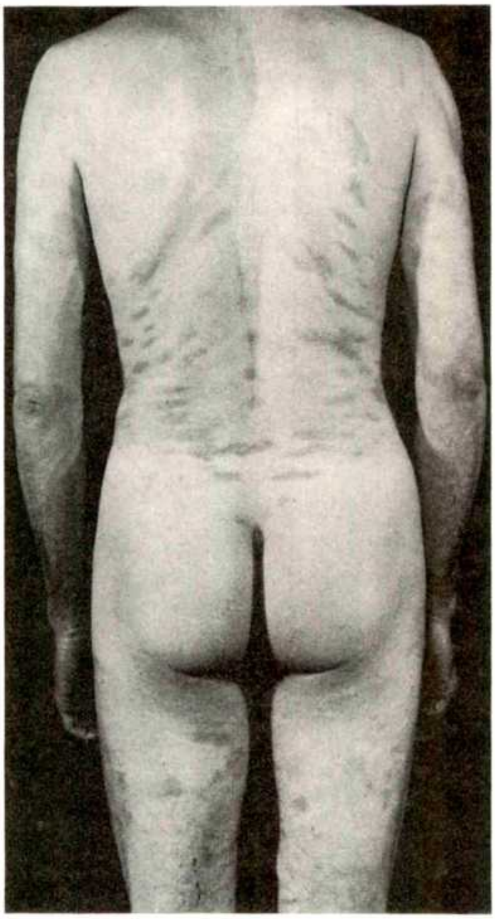

FIG. 1.—Parapsoriasis en plaques.

This condition was given the name xantho-erythrodermia perstans by Radcliffe-Crocker (1905). This emphasizes the yellow colour which is, however, a feature of rather a small number of cases; in most the colour is pink. Radcliffe-Crocker knew of the possible association with mycosis fungoides, but considered his 9 cases to be benign. Calnan and Meara (1956) used the name chronic superficial dermatitis for some of these cases in which the limbs were predominately affected. They recognized their cases as benign. As the histological changes are those of a mild eczema, this term might be used to cover all cases. Montgomery (1967) called the condition the simple discrete type of parapsoriasis en plaques. He, too, recognized it as benign.

During the past 10 years a register has been kept at St John's Hospital of patients with conditions which might be regarded as potentially prereticulotic or as frankly reticulotic. These have included cases of parapsoriasis en plaques or chronic superficial dermatitis and, indeed, they form the largest single group. Previous reports have been made in 1964 and 1968 (Samman, 1964, 1968). It soon became apparent that these cases behaved differently from most other conditions included in the register. Included in the group are only those cases in which the clinical and histological appearances supported the benign nature of the condition. A few cases which at the outset are clinically parapsoriasis en plaques develop in the first 2 or 3 years a reticulate pattern in some patches, and these are included in the poikiloderma group. Likewise, in a few cases the original histology was more in keeping with a prereticulotic condition, and these too are included in the poikiloderma group. There are a total of 7 cases in these 2 categories.

TABLE I.—*Parapsoriasis en Plaques. Age at Onset*

	Number of Cases	
Age at Onset	Male	Female
0–10	0	0
11–20	8	1
21–30	17	3
31–40	34	7
41–50	46	13
51–60	32	4
61–70	10	4
71–80	1	0
Total	148	32

Table I contains all 180 cases included in the register at the time of writing. It will be seen that the onset is usually in the middle years of life and that no case started under the age of 10 years. Men are affected more than 4 times as often as women. These cases were almost all Caucasian, but probably no race is exempt. The condition appears to be more common in the south than in the north of England.

TABLE II.—*Parapsoriasis en Plaques. Progress of the Disease*

	Number of Cases	
Status	Male	Female
Dead	6	2
Lost from follow-up	16	4
Discharged clear	8	7
Persisting without great change:		
8–10 years	9	0
11–15 years	14	6
16–20 years	13	1
Over 20 years	10	4
Total	76	24

Table II is based on the first 100 cases in the register. All of these have had their disease 8 years or longer. The 8 deaths were all from intercurrent disease and in no way related to their skin condition. The potential to clear permanently is small, but higher in women than in men. Excluded from this group are 7 cases for reasons already stated, but none of these 7 has yet progressed to a reticulosis. In my first report (Samman, 1964) one patient in this group showed signs of progress to a reticulosis. This patient died of carinoma of the lung without further progress of his skin disease. Two others developed a reticulate patterning (Samman, 1968) soon after the onset of their disease and are included in the 7 mentioned above, and are now transferred to the poikiloderma group.

POIKILODERMA ATROPHICANS VASCULARE; PRERETICULOTIC POIKILODERMA OR ATROPHIC PARAPSORIASIS

The pre-malignant form is usually referred to in this country as poikiloderma atrophicans vasculare, but elsewhere is often called parapsoriasis, lichenoid parapsoriasis or parapsoriasis en plaques. The patches are usually larger than in the benign type; they are most often found in the major flexures, on the buttocks, and in women over the breasts. They vary in extent from a few small or larger patches to almost complete involvement of trunk and limbs. The patches are erythematous, slightly wrinkled and with minimal scales. They have a reticulate appearance made up partly by a network of pigment and partly by slight atrophy. There are few symptoms, but the patient may complain of a feeling of dryness and discomfort in cold weather. As with the benign type, the condition is very stubborn, but response to u.v. light or natural sunlight is more permanent when it

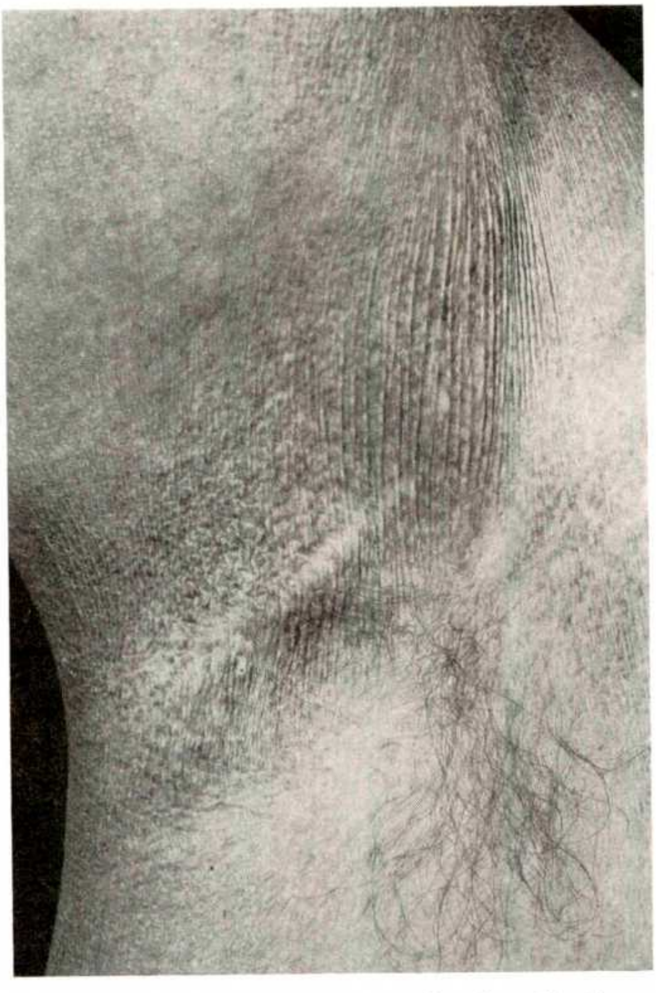

FIG. 2.—Prereticulotic poikiloderma.

occurs. After a time, in a minority of cases, some patches become irritable and may become infiltrated. Progress to a frank reticulosis is uncommon. Montgomery (1967) calls this the large plaque type of parapsoriasis en plaques; and Palmer in his thesis prefers atrophic parapsoriasis. The histology is characteristic. There is some epidermal atrophy. Close up under the epidermis is an intense infiltrate consisting mainly of lymphocytes, but with some reticulum cells and some abnormal cells. The lower margin of the infiltrate is usually well defined, and there is often some invasion of the epidermis by mononuclear cells.

TABLE III.—*Prereticulotic Poikiloderma. Age at Onset*

	Number of Cases	
Age at Onset	Male	Female
0–10	7	3
11–20	5	5
21–30	12	7
31–40	14	9
41–50	16	7
51–60	14	5
61–70	2	1
71–80	0	0
Total	70	37

Table III includes all 107 cases included in the register at the time of compilation of this report. The condition is a good deal less common than the benign type, especially in the south of England. The age of onset is often younger, and 10 cases started in the first decade of life. The preponderance of men is less than in the benign type.

TABLE IV.—*Prereticulotic Poikiloderma. Progress of the Disease*

	Number of Cases	
Status	Male	Female
Dead	2	3
Lost from follow-up	7	3
Discharged clear	2	2
Persisting without great change:		
8–10 years	3	1
11–15 years	2	1
16–20 years	6	4
Over 20 years	6	5
Progress to mycosis fungoides or reticulosis	2	1
Total	30	20

Table IV is based on the first 50 cases in the register. All have had their condition for 8 years or more. They include the 7 cases mentioned earlier as excluded from the parapsoriasis group. In this group 2 of the deaths were directly related to their skin disease. One died of leukaemia and one of mycosis fungoides. The skin histology in the later stages of the latter was that of a lymphosarcoma, but there was no evidence of internal involvement. It will be seen that, even in this group, the potential of becoming frankly malignant is small. There is a very definite chance that some patches will clear permanently, either spontaneously or with treatment: u.v. light or natural sunlight are most helpful in this respect. Poikilodermatous changes are, however, seen so frequently in advanced mycosis fungoides that prognosis must always be guarded when prereticulotic poikiloderma is encountered. There is no doubt that more of these cases will eventually become malignant, but progress is very slow. From my earlier reports it might have been expected that more than 5 of the first 50 cases would have become frankly malignant by now. In over half the cases the histology pointed to a progression to malignancy, but that this has not occurred suggests that the body can maintain the *status quo* for a long time, or even reverse the trend to malignancy.

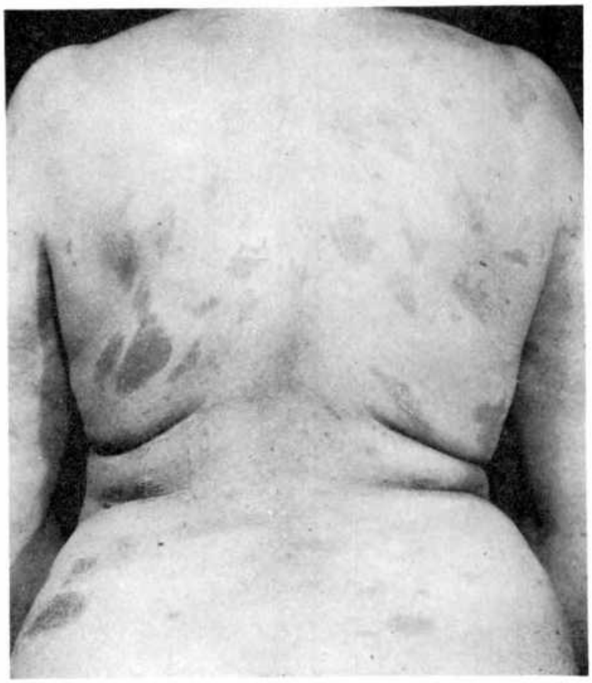

FIG. 3.—Early mycosis fungoides. (note variations in colour of plaques)

WHY HAS PARAPSORIASIS EN PLAQUES SUCH A BAD NAME?

This is largely a question of nomenclature. The term should include only one disease, but at different times the same name has been applied to 2 or more conditions which are not clearly separated in the literature. The benign condition is probably much commoner in England than elsewhere, and it is possible that some people call it eczema rather than parapsoriasis. That both conditions described here are called parapsoriasis en plaques is shown by the Degos' text " Dermatologie " in which each is illustrated over the title " Parapsoriasis en Plaques ". In the literature cases which progress to a reticulosis are more likely to be reported than those which remain benign. Keil (1938) collected a large number of these references.

There are 2 other factors which have led to confusion. The first has already been noted, namely that, in the earliest stages of the prereticulotic form, the benign type may be closely simulated, but the reticulate patterning is usually visible within the first 2 or 3 years. A biopsy will often give an earlier warning in these cases. The second is that the early clinical changes of the classical Alibert type of mycosis fungoides can closely resemble parapsoriasis en plaques, but the patches are less uniform in colour and shape and the histology is almost certain to be different. The illustration will, I hope, show these differences. To prevent continuing confusion it is obvious that some internationally agreed nomenclature is needed.

I wish to thank the Department of Medical Illustration, St John's Hospital for the photographs, and numerous colleagues who have referred patients to me and made this investigation possible. Some financial help has been obtained through the scheme for locally organized clinical research in the N.H.S.

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PULMONARY EOSINOPHILIA ASSOCIATED WITH VASCULITIS AND EXTRA-VASCULAR GRANULOMATA

A CASE REPORT AND REVIEW OF THE LITERATURE

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Summary.—An unusual case of necrotizing vasculitis is reported, in which chronic bronchitis preceded the onset of " allergic " vasculitis of the skin, rhinitis, conjunctivitis, pulmonary infiltrates, marked blood eosinophilia and extra-vascular granulomatous proliferation. The case had features of both Churg's allergic granulomatosis and Wegener's granulomatosis, but it was typical of neither condition. The relationship between benign pulmonary eosinophilia, Churg's allergic granulomatosis and Wegener's granulomatosis is discussed, and it is suggested that intermediate cases of this type might be called Chegener's granulomatosis. A review of the literature suggests that the range of syndromes could be extended to include Chöffler's pulmonary eosinophilia, comprising cases transitional between Löffler's and Churg's syndromes. For the sake of brevity and onomatopoeia the Löffler–Chöffler–Churg–Chegener–Wegener syndromes could be linked together as the " Chöffler Train ".

We recently suggested that the conditions characterized by pulmonary eosinophilic infiltration, vasculitis and granulomatous proliferation might form a continuum of syndromes ranging from Löffler's benign pulmonary eosinophilia, through Churg's allergic granulomatosis, to Wegener's granulomatosis (Burton and Burton, 1972). We here report a case which appears to be transitional between Churg's granulomatosis and Wegener's granulomatosis, in that it has features of both conditions, but is typical of neither. A review of the literature has revealed many other cases which might be transitional between Löffler's and Churg's syndromes.

CASE REPORT

History.—The patient, a man, aged 78 presented to the Skin Clinic in April 1971 with an 8-week history of weight loss (6 kg), malaise, stiffness of shoulder muscles and " blood blisters " of the hands and feet. System review revealed a recent episode of colicky abdominal pain lasting 3 days, associated with abdominal distension and followed by the passage of red blood per rectum. There was no history of recent drug ingestion or infection prior to the onset of symptoms. The patient denied any past or present respiratory symptoms, although his general practitioner later reported that he had treated him on several occasions in previous years for " chronic bronchitis " during the winter months. These mild illnesses had been accompanied by cough, with mucopurulent sputum, but no marked bronchospasm.

Examination.—This revealed a thin, ill man with conjunctivitis, serosanguinous crusting of the nares, mouth ulcers and widespread purpuric, urticated lesions and

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haemorrhagic blisters of the extremities suggestive of vasculitis. There were no cutaneous nodules, and livedo reticularis was absent. The only other abnormality noted was gross restriction of mobility of the shoulder-joints, which were painful on active or passive movement (" frozen shoulders ").

Investigations.—On admission to hospital these revealed hypochromic anaemia (Hb 9·1 g/100 ml), with a w.b.c. count of $8300/\text{mm}^{-3}$, of which 24% ($1990/\text{mm}^{-3}$) were eosinophils, and an E.S.R. of 127 mm/hour. Platelets were normal. There was no proteinuria or microscopic haematuria, and other investigations which were normal included x-rays of chest, cervical spine, abdomen and shoulder joints, barium enema, E.C.G., blood urea and creatinine clearance, liver function tests, faecal fats, faecal occult blood, Rose–Waaler, antinuclear factor and Wasserman reaction. Protein electrophoresis revealed a generalized increase in α_1-, α_2-, and γ-globulins. Electromyogram of biceps, deltoid and abductor pollicis brevis showed scanty fibrillation potentials suggestive of mild diffuse denervation.

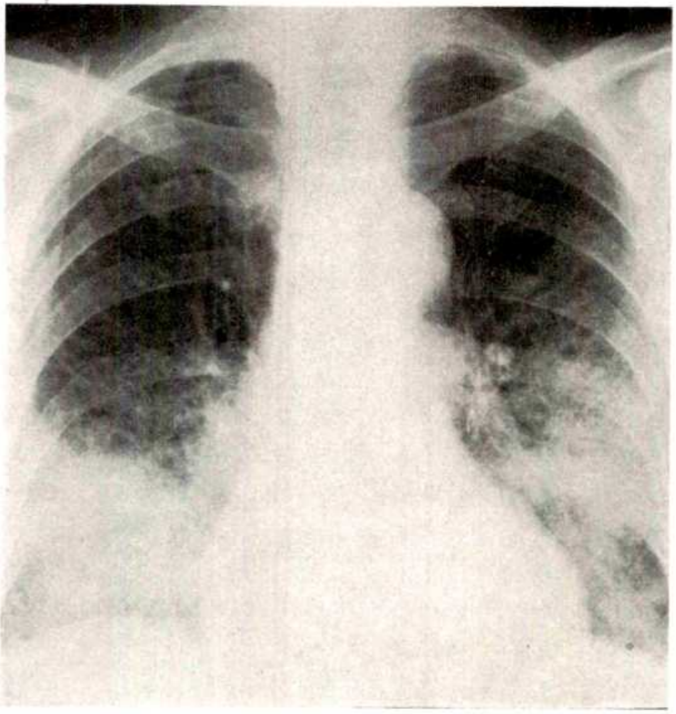


FIG. 1.—Chest x-ray, showing widespread patchy shadowing compatible with pulmonary eosinophilia.

Progress.—One week after admission to hospital the patient developed a cough, with purulent sputum, and pyrexia with temperature spikes of 39°C. Examination revealed impaired percussion and bronchial breathing at the lung bases, but no rhonchi. Chest x-ray at this time (Fig. 1) showed widespread patchy shadows in the lower half of both lungs. Sputum culture yielded *Staph. aureus*; but, despite treatment with cloxacillin and ampicillin for one week, the patient deteriorated rapidly, although the signs of consolidation cleared. Further chest x-rays showed little change in severity, although the detailed distribution of the diffuse shadows varied. At this time a skin biopsy (Fig. 2) showed a small subepidermal vesicle, with a collection of mononuclear cells in the underlying dermis, composed largely of histiocytes, with occasional Langhans' type giant cells and a few plasma cells. There was a mild perivascular lymphocytic infiltration in the dermis, but no evidence of a vasculitis. Acid- and alcohol-fast bacilli were not seen in appropriately stained sections. In view of this unexpected histology, the clinical diagnosis of polyarteritis nodosa was questioned, and alternative possibilities such as tuberculosis were discussed. Acid-fast bacilli were absent from the sputum, however, and the patient, by now apparently moribund, was treated with prednisone 60 mg daily. The response was remarkably good; and, within 2 weeks, the patient was well, with a normal chest x-ray, E.S.R. 24 mm/hour and a w.b.c. count of 7000, with 2% eosinophils. A second skin biopsy, taken before the steroid was started, showed a small focus of inflammatory cells in the upper dermis. These were mainly neutrophils, with many nuclear fragments and some extravasated red cells. There was no definite evidence of necrosis of vessel walls, but the appearances were suggestive of "allergic vasculitis". The patient has maintained his good progress on decreasing doses of prednisone and remains well 6 months later with a dose of 5 mg b.d.

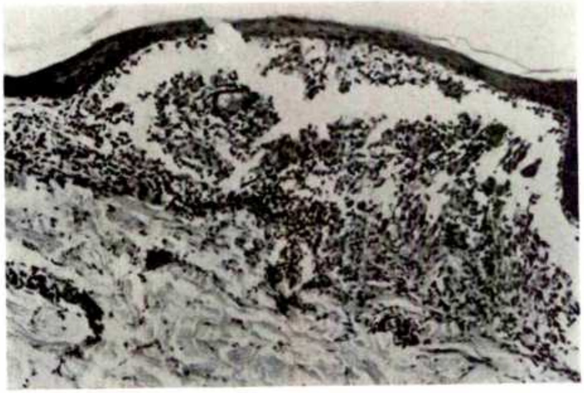


FIG 2.—Skin biopsy, showing subepidermal vesicle with giant cell granulomatous proliferation (H. and E. × 100).

DISCUSSION

Polyarteritis nodosa is characterized by focal necrotizing vasculitis of muscular arteries of the calibre of the coronary vessels. Since the original description (Kussmaul and Maier, 1866), hundreds of cases have been reported, many of which do not conform to this description. Most authorities now accept the suggestion of Zeek (1952, 1953) that true polyarteritis nodosa should be distinguished where possible from its variants, such as Churg's allergic granulomatosis, Wegener's granulomatosis, Zeek's hypersensitivity (leukocytoclastic) angiitis and cranial (giant cell) arteritis.

The present case has features of both Churg's allergic granulomatosis and Wegener's granulomatosis, but is typical of neither. Allergic granulomatosis was recognized as a clinico-pathological syndrome by Churg and Strauss (1951). They described 13 cases (mainly *post-mortem*) characterized clinically by severe asthma, fever and eosinophilia, and histologically by widespread necrotizing vasculitis, with characteristic changes in the vascular and extravascular connective tissue. These changes consisted of fibrinoid collagen with granulomatous proliferation of epithelioid and giant cells. Ten of these patients had haemorrhagic skin lesions or subcutaneous nodules (Strauss *et al.*, 1951), and the characteristic granulomatous changes were present in or beneath the dermis in at least 6 patients. Since their report, the existence of this condition as a consistent syndrome to be distinguished from true polyarteritis nodosa has been confirmed by the large series of cases of necrotizing vasculitis studied *post mortem* by Zeek (1953) and Moskowitz *et al.* (1963). Surprisingly, only 4 cases appear to have been reported *ante mortem* (Lincoln, 1957; Abul-Haj and Flanagan, 1961; Sokolov *et al.*, 1962; Varriale *et al.*, 1964). There are, however, many cases in the literature reported as polyarteritis nodosa which were probably allergic granulomatosis, e.g. Wilson and Alexander (1945); Hennel and Sussman (1945); Hejtmancik *et al.* (1949); Rose and Spencer (1957); Ellman (1958—Cases 1 and 2); Scadding (1958—Case 3). Recent authors have emphasized the importance of asthma accompanied by fever and eosinophilia as the striking initial manifestation of allergic granulomatosis. Braverman (1970a) has pointed out that the asthma precedes the systemic illness by months or years and characteristically remits before the onset of the vasculitic phase. He also records that nearly every reported case has had abdominal pain and bloody diarrhoea, and many patients have arthralgia and peripheral neuropathy. The present case would appear to be an example of Churg's allergic granulomatosis were it not for the absence of any history of asthma, and the presence of nasal ulceration and conjunctivitis.

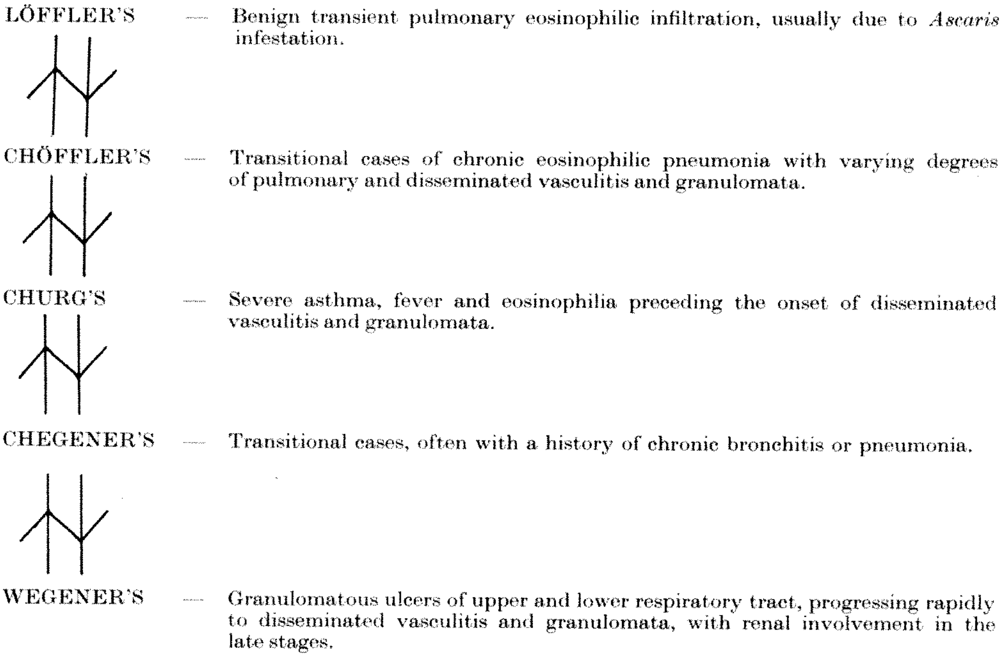
Wegener's granulomatosis (Wegener, 1939) is characterized pathologically by giant cell granulomatous ulcers in the upper or lower respiratory tract, with widespread granulomata (especially in lungs, kidney and spleen) and generalized necrotizing vasculitis (Godman and Churg, 1954). Walton (1958), in a review of the literature, found that cases present insidiously either with upper respiratory tract symptoms such as rhinorrhoea, antral pain and epistaxis or with pulmonary symptoms such as cough, haemoptysis or pleurisy. They pursue a rapidly downhill course, with widespread mucosal ulceration and necrosis in the nose and palate, and pulmonary consolidation. Renal involvement, indicated by albuminuria, haematuria or uraemia, occurs in the late stages of nearly every case. Arthralgia, conjunctivitis and peripheral neuropathy are common, and nearly half have eosinophilia. Reed *et al.* (1963) reviewed the cutaneous manifestations of 118 reported cases of unequivocal Wegener's granulomatosis and found that 51% had cutaneous signs. In the early stages there are haemorrhagic vesicles and papulonecrotic lesions of the extremities, or pyoderma gangrenosum. Advanced cases have generalized exanthemata, often purpuric, but this may be partly attributable to uraemia. Subcutaneous nodules and livedo reticularis do not occur in this condition. Wegener's granulomatosis has many features in common with allergic granulomatosis, but is distinguished by the aggressive nature of the upper respiratory tract granulomata, the regular occurrence of renal lesions and the absence of asthma. Walton (1958) has pointed out that upper respiratory tract ulceration does not occur in allergic granulomatosis, and the presence of nasal crusting, mouth ulcers and conjunctivitis in the present case, together with the absence of asthma, would support the diagnosis of Wegener's granulomatosis rather than allergic granulomatosis. The absence of renal involvement and the rapid resolution of the upper respiratory mucosal lesions following steroid therapy militate strongly against this suggestion, however.

The group of conditions characterized by necrotizing vasculitis (formerly known as polyarteritis nodosa) defies rigid classification. The 2 most useful current classifications are those of Zeek (1953) and that which distinguishes cases according to the size of artery involved. Rose and Spencer (1957), in a retrospective study of 111 cases of " polyarteritis nodosa ", found that neither classification applied satisfactorily to their series, but their cases fell into 2 clear groups characterized by the presence or absence of lung involvement. In the majority of cases the lungs were completely spared, even when there was severe polyarteritis in almost every other organ, but the 32 cases with pulmonary involvement formed a distinctive subgroup characterized by marked blood eosinophilia, granulomatous lesions in the lungs and other viscera, and a distinctive polyarteritis which was eosinophilic in the early stages and granulomatous, sometimes with giant cells, during the healing stage. Other distinctive features in this group were nasal granulomata or rhinitis, mucosal ulcers of the alimentary tract, and a history of respiratory symptoms such as bronchitis, asthma or recurrent pneumonia, which sometimes preceded the onset of the systemic illness by several years. Rose and Spencer recognized that many of their cases of " pulmonary polyarteritis nodosa " were examples of either Churg's allergic granulomatosis or Wegener's granulomatosis, but their series contained a number of cases which could not be assigned to either category. It is clear that the present case falls into this " pulmonary " subgroup and is transitional between allergic granulomatosis and Wegener's granulomatosis. Godman and Churg (1954) described a similar transitional case, and Braverman (1970b) mentions 3 other cases which appear to fit into this category.

The clinical and radiological findings in the present patient were compatible with an eosinophilic pneumonia. The rapid resolution of these changes following steroid therapy suggested also that the changes were due to eosinophilic infiltration rather than pulmonary arteritis.

Several previous authors (Bergstrand, 1946; Smith, 1948; Ehrlich and Romanoff, 1951; Sokolov *et al.*, 1962) have suggested a possible relationship between widespread necrotizing vasculitis with pulmonary involvement and benign, transient eosinophilic infiltration of the lungs, which they called " Löffler's syndrome ". A high proportion of the cases originally described by Löffler (1932) were associated with *Ascaris* infestation, and the term " Löffler's syndrome " is now reserved by some authorities for this aetiologically distinct group. Many cases of benign pulmonary eosinophilia, however, are of unknown cause and have a much less well-defined course. Autopsy reports on cases of benign pulmonary eosinophilic infiltration are rare, but Meyenberg (1942), in 4 subjects killed in accidents, found granulomata in one case, and eosinophilic infiltration of organs other than the lungs in 3 other cases. Numerous cases have been reported which may represent transitional forms between this benign syndrome and Churg's granulomatosis. Zuelzer and Abt (1949) reported a syndrome of children, characterized by eosinophilic infiltration of the viscera. Some cases had focal necrosis with giant cell granulomata, but vasculitis was absent. Carrington *et al.* (1969) described 9 cases of chronic life-threatening eosinophilic pneumonia, some without asthma, which responded dramatically to steroid therapy. Two of their cases had giant cell granulomata on lung biopsy, and another had subcutaneous nodules, which on biopsy revealed a micro-angiitis. Fienberg (1953) has described a patient with asthma, eosinophilia, and vasculitis and granulomata confined to the lungs. Harkavy (1943) has described cases of chronic asthma with eosinophilia and vasculitis, but no focal granulomata. Bergstrand (1946) also described 4 cases of fatal asthma, with pulmonary eosinophilia and vasculitis, and granulomata confined to the lungs.

Ehrlich and Romanoff (1951) reported the case of a patient who died of asphyxia 10 days after his first attack of asthma. He had a blood eosinophilia, and autopsy revealed eosinophilic infiltration of the lungs, gastro-intestinal tract, spleen and diaphragm. There was necrotizing vasculitis of some arteries at the edge of the pulmonary infiltrates, and granulomata were present in the lungs. Bayley *et al.* (1945) described a similar case of fatal eosinophilic pneumonia associated with asthma and blood eosinophilia, in which autopsy showed focal pulmonary granulomata and necrotizing vasculitis. Smith (1948) also described a case with pulmonary eosinophilia, asthma and vasculitis affecting the lungs, kidneys and spleen. This case borders on Churg's syndrome, but the granulomatous areas were confined to the lungs.



LÖFFLER'S — Benign transient pulmonary eosinophilic infiltration, usually due to *Ascaris* infestation.

CHÖFFLER'S — Transitional cases of chronic eosinophilic pneumonia with varying degrees of pulmonary and disseminated vasculitis and granulomata.

CHURG'S — Severe asthma, fever and eosinophilia preceding the onset of disseminated vasculitis and granulomata.

CHEGENER'S — Transitional cases, often with a history of chronic bronchitis or pneumonia.

WEGENER'S — Granulomatous ulcers of upper and lower respiratory tract, progressing rapidly to disseminated vasculitis and granulomata, with renal involvement in the late stages.

FIG. 3.—The " Choffler Train "—syndromes characterized by varying degrees of pulmonary eosinophilia, necrotizing vasculitis and granulomatous proliferation.

These cases suggest the possibility that there may be a range of syndromes from benign transient pulmonary eosinophilia (which many authors label " Löffler's syndrome "), through chronic eosinophilic pneumonia and cases with a higher incidence of vasculitis and extra-vascular granuloma formation, to the full-blown Churg's syndrome.

The range of cases we have described in this review are thus characterized by varying degrees of pulmonary eosinophilic infiltration, granulomatous proliferation and necrotizing vasculitis. There is some evidence that immunological hypersensitivity may be the common pathogenetic factor relating these conditions, and the resulting clinico-pathological syndrome may depend, for example, on such variables as the size and site of vessels involved, the extent of eosinophilic infiltration, and the degree of necrosis and granulomatous reaction provoked.

A plethora of terms has been used to describe the various entities which may be included in this group, but it is difficult to find a single term to embrace the whole range. Fienberg (1955) has discussed these difficulties of nomenclature, and he introduced the term " pathergic granulomatosis ", pathergy being defined as the totality of the morbid phenomena which can be produced by a state of altered reactivity. This term does not appear to have become widely adopted in the literature, however, and we recently proposed (Burton and Burton, 1972) that, by the hybridization of eponyms, this range of conditions might be more memorably identified as the " Löffler–Chöffler–Churg–Chegener–Wegener syndromes ", which might for the sake of brevity and onomatopoeia be linked together as the " Chöffler Train ". Until advances in our knowledge of the aetiology of these conditions results in a rational resolution of the present imbroglio, we feel that the scheme we have outlined (Fig. 3) may serve to emphasize the possible relationship between these syndromes, and the fluidity of their boundaries.

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THE AETIOLOGY OF IMPETIGO CONTAGIOSA

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Summary.—The study entailed bacteriological investigation of 131 cases of impetigo contagiosa, including the isolation and identification of the offending organisms. The data showed the presence of *Staphylococcus aureus* in pure culture in 40·5% of the cases, and in mixed growth with betahaemolytic streptococci and other organisms in 47% of the cases. Streptococci came next in frequency and were isolated in pure culture from 9·1% of cases, and in combination with other organisms in 36·7% of the cases. A haemolytic diphtheroid having the characteristics of *Corynebacterium pyogenes* was isolated in pure culture from one case and in mixed growth in 5·3% of the cases.

IMPETIGO contagiosa may be defined as an acute superficial infection of the skin, vesicular at the onset and crusted later, which heals without scar formation.

Our present knowledge of impetigo contagiosa (I.C.) extends from its first description as a separate clinical entity by Tilbury Fox in 1864, and the proof of its bacterial aetiology by Radcliffe-Crocker in 1881.

The initial lesion in impetigo is a very thin walled vesicle on an erythematous base. The vesicles rupture so rapidly that they are seldom seen as such. The exuding serum soon dries to form yellowish-brown crusts. Gradual peripheral extension occurs, and multiple lesions may coalesce. The crusts eventually dry and separate to leave an erythema that fades gradually. The face is the site of predilection, although the neck, hands and other parts of the body, except the palms and soles, are occasionally attacked. The lesions may appear singly or in crops. Children are more susceptible to the disease than adults.

Both staphylococci and streptococci have been recovered from the lesions, but the relative preponderance of each varies greatly, and reports are contradictory. Differences in the clinical characteristics related to staphylococci or streptococci in relation to the thickness of the vesicle wall have been described (Tachau, 1938). The streptococcal disease manifests as thin fragile blebs with wide areolae; the staphylococcal disease presents thicker walled, more deeply seated lesions, which are often associated with follicular or furuncular elements. The association of significant regional lymphadenopathy with streptococcal impetigo was noted by Burnett (1962). Marcowitz *et al.* (1965), however, found no difference in the incidence of lymphadenopathy among children with lesions positive for streptococci, staphylococci, or negative for both organisms.

Evans (1941) suggested the existence of a specific type of Gram-positive cocci, for which he postulated the name " impetigococcus ".

Arndt (1965) postulated that impetigo may prove to be an example of bacterial synergism in man in which both staphylococci and streptococci work together to induce this peculiar type of skin infection.

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Staph. aureus was considered to be the most important pathogen in this condition by many investigators, e.g. Bigger and Hodgson (1943); Sheehan and Fergusson (1943); Parker *et al.* (1955); Barrow (1955); van Toorn (1961).

On the other hand Swift (1952) believed that haemolytic group A streptococci are the primary aetiological agents and that staphylococci are only secondary invaders. Dillon (1968) supported this view.

Staph. aureus type 71 and other group II staphylococci have predominated in many cases (Barrow, 1955; Parker *et al.*, 1955; Van Toorn, 1961; Brundin and Laurell, 1963). Type 80/81 has predominated in other cases (Naylor and Rook, 1968).

Parker *et al.* (1955) were the first to demonstrate that certain serotypes of streptococci were more often associated with impetigo than with infection at other sites, and postulated the existence of impetigo strains. Most of the strains were group A streptococci with agglutination complexes 3/13/B3264, 8/25/Imp 19, 5/11/12/27/44 or 25/Imp 19; other types were not so commonly found. These findings were later confirmed (Dillon *et al.*, 1965, 1967; Dillon, 1968).

MATERIALS AND METHODS

One hundred and thirty-one cases of impetigo contagiosa were studied. Most of the patients were infants and children. Their age distribution was as follows:

0–1 year	24 cases
1–5 years	80 cases
5–12 years	27 cases

Sixty-eight were females and 63 were males. Most of the patients had face or scalp lesions, but lesions elsewhere were also seen.

Sheep's blood agar plates were taken to the outpatient clinic of the Cairo University paediatric hospital, and samples from the patients were inoculated directly.

Early cases with unbroken vesicles were chosen whenever possible, but these constituted only a minority of the cases examined (10 of 131); and, in most instances, we were confronted with already crusted lesions. Cases giving a history of recent antibiotic therapy were excluded.

In unbroken vesicles or pustules the skin over the lesion was cleansed with acetone, left to dry and then punctured with a sterile needle; the fluid was directly smeared on the plates of blood agar, using a bacteriological loop. When dealing with crusted lesions, the crust was removed with a sterile forceps, and sterile cotton swabs moistened in broth were rubbed on the base of the lesion and used for inoculating the blood agar plates.

Two plates were used for each specimen, one incubated aerobically and the other under anaerobic conditions in a McIntosh and Fildes jar. The plates were examined after 24 hours and 48 hours incubation, and any growth was isolated in pure culture and identified.

No obligatory anaerobes were encountered, and isolates from aerobic and anaerobic cultures were the same.

From preliminary morphological studies only 3 types of organisms could be recognized, namely, staphylococci, streptococci and diphtheroids.

Staphylococci were identified by their morphology, pigment production on milk agar, and the haemolytic activity on blood agar plates. The slide clumping test was used as a screening test for coagulase production by isolated strains (Cadness-Graves *et al.*, 1943). Colonies giving positive results and those showing doubtful reactions were re-examined by the tube coagulase test using 1 : 5 dilution of fresh citrated human plasma (Fisk, 1940). Twenty-five of the isolated and identified *Staph. aureus* strains were phage typed.

Streptococci were identified by their morphology and by the changes they produced in blood agar. A primary grouping based on bacitracin inhibition was used to identify group A strains (Maxted, 1953). Further grouping according to Lancefield (1933) was carried out by a precipitation test, using an acid extract of the group specific polysaccharide antigen against group specific sera, and performed in capillary tubes. Type identification within group A strains was carried out by the slide agglutination method.

Members of the corynebacterium group were recognized by their microscopic morphology. Strains were further identified on the basis of haemolysis on sheep's blood agar plates, sugar fermentation, and some other biochemical tests, including litmus milk reaction and liquefaction of gelatin. Pathogenicity tests were done by subcutaneous inoculation of the isolated cultures in rabbits and by intraperitoneal injection in guinea-pigs.

RESULTS

Staph. aureus was isolated from 114 cases (87%). The organism was isolated in pure culture from 53 cases (40·5%) and in combination with streptococci from 47 cases (36%); in 12 cases (9·1%) it was present together with a diphtheroid. In only one case *Staph. aureus*, *Strep. pyogenes* and a haemolytic diphtheroid were present together; and, in another case, *Staph. aureus* was recovered in combination with a coagulase-positive *Staph. albus*. A haemolytic coagulase-positive *Staph. albus* was isolated in pure form from one case. Phage typing was done on only 25 of the *Staph. aureus* strains isolated in pure culture from unbroken vesicles or crusted lesions. The strains belonged to group I and II, and were distributed as follows:

Type 80	16 strains
Type 52/80/81	2 strains
Type 52/80	1 strain
Type 71	1 strain
Untypeable	5 strains

Streptococci were isolated from 60 cases (45·8%); betahaemolytic strains were present in 58 cases (44·2%); 2 strains were non-haemolytic. A pure culture of betahaemolytic streptococci was obtained from 12 cases (9·1%). In the remaining cases it was present with *Staph. aureus*. Betahaemolytic streptococci were grouped and typed and were found to belong to the following groups:

Group A	37 strains	72%
Group C	4 strains	8%
Group G	8 strains	16%
Group D	2 strains	4%

Seven strains were not typed. Group A haemolytic streptococci (*Strep. pyogenes*) comprised the following types:

Type 9	4 strains
Type 14	2 strains
Type Imp 19	11 strains
Type 23	6 strains
Type 5/27/44	12 strains

Two strains were auto-agglutinable and could not be typed.

A haemolytic diphtheroid was isolated from 7 cases (5·3%). In one case it was obtained in pure culture; in another case it was combined with *Strep. pyogenes* and *Staph. aureus*. In the remaining 5 cases it was obtained together with *Staph. aureus*. Non-haemolytic diphtheroids were isolated in combination with *Staph. aureus* from 7 cases (5·3%). The corynebacterium strains haemolytic on blood agar were of a beaded morphology and contained few Neisser granules. They were found to ferment glucose, mannitol, maltose, sucrose and lactose; to produce an acid clot on litmus milk and to liquefy gelatin slowly. Subcutaneous injection of these strains into rabbits produced a localized, indurated swelling which slowly softened to a necrotic abscess. Intraperitoneal injection into male guinea-pigs killed the animal in 2–4 days, and the organisms were recoverable from the liver and spleen, but not the heart blood of the animals. *Corynebacterium pyogenes* is known to produce suppurative lesions in animals, and our strains closely resembled this organism. The 7 non-haemolytic diphtheroid strains proved to be non-pathogenic to rabbits or guinea-pigs, and were most probably ordinary normal skin diphtheroids contaminating the crusted lesions. In 3 cases no growth was obtained on blood agar plates.

DISCUSSION

Reviewing the available literature, we found discrepancy in the data given by different investigators. Thus, while some workers, e.g. Swift (1952) and Dillon (1968), stressed the significance of streptococci in the pathogenesis of this condition, others (Engman, 1901; Parker *et al.*, 1955; Barrow, 1955; Van Toorn, 1961) emphasized the frequency and importance of staphylococci as the leading aetiologic agents.

Our findings more or less agree with those of Parker *et al.* (1955) and Barrow (1955) in stressing the frequency of *Staph. aureus* in association with *Strep. pyogenes*. The most prevalent phage type among the limited number of strains typed was type 80. Type 71, which was the prevalent type in the strains isolated by Barrow (1955), was only rarely encountered (one of 20 typeable strains).

Organisms resembling *C. pyogenes* have been recovered from the skin lesions. This appears to be a new finding, although Bacon and Marples (1955) and Markham and Stenhouse (1959) have previously recorded *C. diphtheriae* from similar lesions. In the present series most of the children came from rural areas, and it is probable that the sources of infection in these cases were infected animals.

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A ROSACEA-LIKE ERUPTION OF CHILDREN

J. A. SAVIN, SUZANNE ALEXANDER AND R. MARKS

Summary.—Eleven children have shown similar changes on the facial skin. A bright malar flush was accompanied at times by small superficial papules, pustules and scaling. The clinical picture was variable, sometimes closely resembling rosacea and sometimes peri-oral dermatitis, taking an average of 1 year to clear.

Seven of the 11 children had a family history of atopy, and 2 had a family history of rosacea. Potent topical corticosteroids may have played a role in the cause of these children's rash and, on withdrawal, there was sometimes a pustular exacerbation. The relationship to rosacea and peri-oral dermatitis is discussed.

WE wish to draw attention to 11 children who have shown similar changes on the facial skin. The main features were a bright malar flush with, at times, the accompaniment of small superficial papules, pustules and scaling; in older patients this would have suggested a diagnosis of rosacea.

PATIENTS

Details are given in Table I. The flush was persistent and bright red. It was usually not sharply marginated, and affected the cheeks, although occasionally the forehead and chin were involved. The erythematous areas were often studded with small papules and superficial pustules (Fig. 1, 2). In 3 patients small hemispherical papules were scattered around the eyelids (Fig. 3). However, the picture was variable and occasionally resembled perioral dermatitis (cf. Fig. 2, 4). Sometimes scaling was quite prominent (Fig. 5).

TABLE I.—*Details of Patients with a Rosacea-like Eruption*

Patient no.	Sex and age at onset (years)	Past history of eczema	Family history of atopy	Keratosis pilaris	Onset to cure (months)
1	F 12	+	+	—	Still present
2	M 2	+	+	—	6
3	M 1	—	+	—	24
4	F 5	—	—	+	6
5	M 5	+	—	—	Not known
6	F 3	—	+	—	15
7	F 3	—	+	+	36
8	M 2	—	+	—	5
9	M 7/12	—	—	+	12
10	M 1	—	+	—	6
11	F 4	—	—	—	3

The parents emphasized that the condition was not itchy, but despite this, at least 8 were known to have been treated with potent topical corticosteroids (betamethasone-17-

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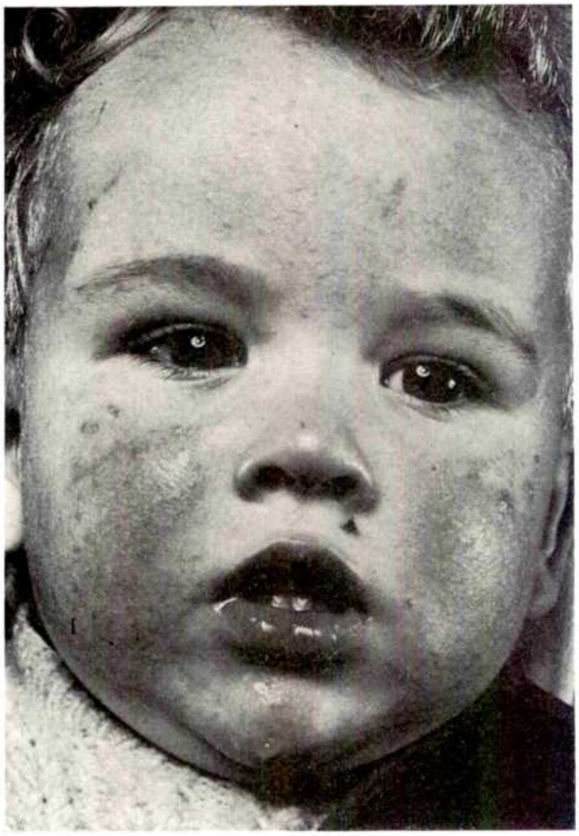
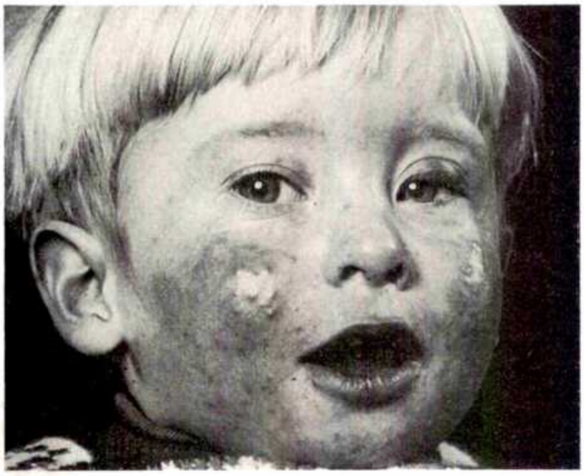


FIG. 1 and 2.—The skin changes showing a fixed malar flush with small papules and superficial pustules.



valerate, 7 patients; fluocinolone acetonide, 1 patient) for many months before attending the hospital skin clinic. An exacerbation with increased redness, scaling and pustulation was often noted on withdrawal of these preparations (Fig. 6). After this, other creams were well tolerated, and the trend was towards a slow but complete remission, which sometimes took several months to achieve (average 1 year) (Table I).

Of the 11 children, 7 had a history of atopy in their immediate family and 3 had themselves had infantile eczema. Three children had marked keratosis pilaris on the arms, but no other evidence of ichthyosis. The mothers of 2 patients were noted to have had rosacea.

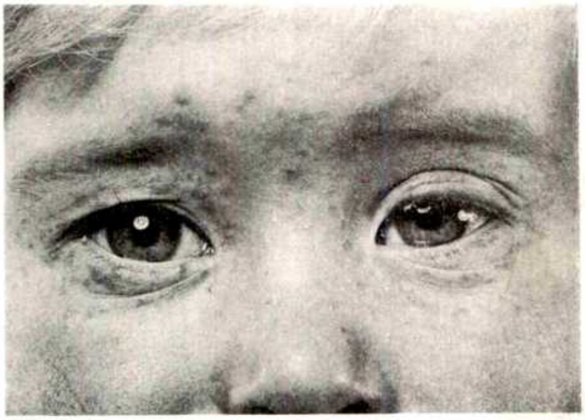


FIG. 3.—A scattering of small papules around the eyelids.

Detailed Case Histories

Patient 3.—An otherwise fit boy aged 2 years presented with a one-year history of an eruption which had started at the corner of the mouth, but which spread to involve both cheeks. Topical treatment with betamethasone-17-valerate had not cured the eruption although it had been applied over a period of several months. There was a strong family history of asthma on his father's side of the family.

When first seen, a patchy erythema with a scattering of small pustules was noted on the cheeks and around the mouth. His betamethasone application was stopped and 1% sulphur in calamine liniment was substituted. Three days later he developed acute "flare up" of the rash and was seen again by another doctor as an emergency. Betamethasone was re-introduced and his mother continued to apply this for a further 3 weeks. At the end of this

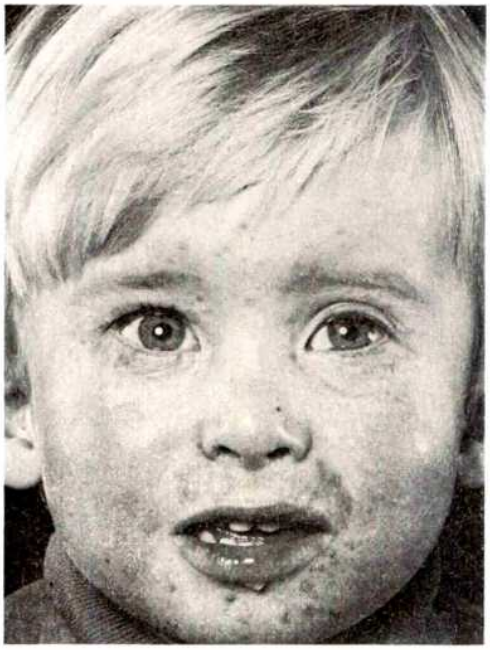


FIG. 4.—The clinical pictures sometimes resembled perioral dermatitis. Compare with Fig. 2.

time his eruption was more rosaceous than ever and the betamethasone was replaced by 0·25% sulphur in aqueous cream. The skin condition then started slowly to subside and, 12 months after he had first been seen, his skin had completely healed.

Patient 4.—Dry skin was noted in early childhood. This was treated by her general practitioner with topical betamethasone 17-valerate. At the age of 3 she was noted to have an erythematous eruption of the forehead, cheeks and chin. Papules, pustules and a few comedones were noted.

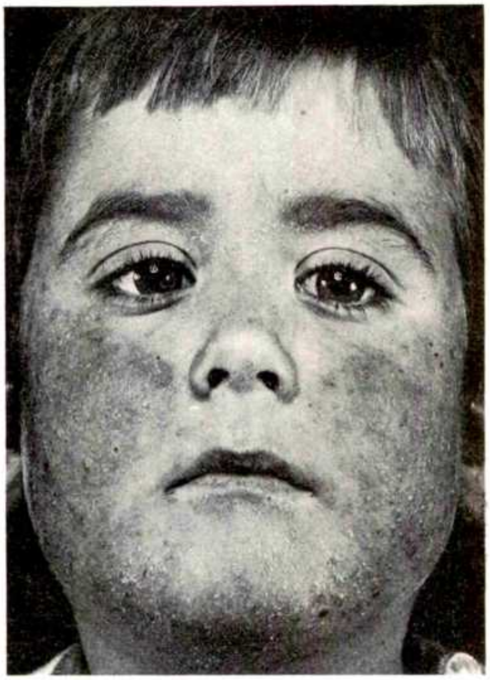


FIG. 5.—Sometimes scaling was quite prominent.

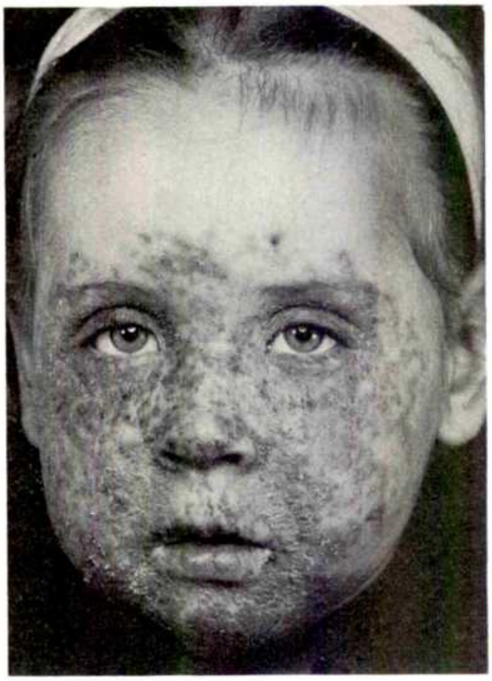


FIG. 6.—An acute exacerbation after the withdrawal of topical steroids. Markedly increased pustulation and scaling.

At that stage demodices were found in scrapings in moderate numbers on one occasion only. Cultures for pathogenic bacteria and fungi were negative. Urinary 17-ketosteroid levels were normal.

Two months after discontinuing betamethasone 17-valerate her skin had returned to normal, although the keratosis pilaris persisted on the upper arms. During this time she had received systemic ampicillin and then systemic oxytetrocycline. Topical treatments had included ichthyol, sulphur and hydrocortisone.

Investigations

Pustules from 7 of the children were cultured for bacterial pathogens; one patient's swab yielded scanty *Staphylococcus aureus*, but no pathogens were isolated from the swabs of the other 6. Monilia and dermatophytes were not isolated from any of these children. *Demodex folliculorum* was looked for in 7 patients, but was found in moderate numbers in only 2.

DISCUSSION

The changes described in these children differ from infantile acne, in which a facial flush is not prominent, by the absence of comedones and deep pustules. Despite the presence of keratosis pilaris on the arms of 3 patients, the changes on the face were not those of keratosis pilaris rubra atrophicans—slight residual pitting was seen on the face of only one patient. The " lupus erythematous-like eruption in infants " (Ive and Sanderson, 1964) differs in that it is light-provoked and not pustular. In none of our patients were there any general symptoms suggestive of dermatomyositis or systemic lupus erythematosus. We know of no exactly similar cases in the literature.

Our title has been chosen to be descriptive rather than to imply a relationship with rosacea as seen in adults; nevertheless several dermatologists who saw these patients remarked on the clinical similarity to rosacea. One patient (No. 4) was presented at a clinical meeting (Pope, 1970) as a possible case of juvenile rosacea. In the discussion which followed (Moynahan, 1970) the skin changes were attributed to the " rebound phenomenon " which may follow the use of fluorinated steroids on children's faces. Certainly we were impressed both with the frequency with which potent topical corticosteroids had been used in our cases and by the pustular exacerbations seen on discontinuing them. Similar exacerbations have been noted in rosacea (Sneddon, 1969a) and in peri-oral dermatitis (Sneddon, 1969b), although this does not necessarily imply that these conditions are related either to each other or to the rosacea-like eruption of children being described here.

We tentatively suggest that rosacea-like eruptions of children are added to the growing list of iatrogenic disease caused by misuse of the potent topical corticosteroids. A possible sequence of events is as follows: the steroid applications are prescribed for a banal eczema of the face or pityriasis simplex; the condition deteriorates due to the fluorinated corticosteroid content; the cause of the deterioration is not recognized and more is prescribed. It is hoped that this report will stop this vicious circle in some cases by producing awareness of this possibility.

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PERIORAL DERMATITIS

IAN SNEDDON

Summary.—Seventy-three patients, 5 men and 68 women, suffering from perioral dermatitis have been seen in the last 2 years in the Rupert Hallam Department of Dermatology, Sheffield. All but 2 had used fluorinated steroids during the course of their illness. Substitution of hydrocortisone for the fluorinated steroid was followed by improvement in some, but the majority of patients required a course of oxytetracycline 250 mg twice daily for several months to produce complete recovery. A postal follow-up of 20 patients who had discontinued treatment for 6–14 months gave 13 useful replies. Eleven patients had had no further skin eruption, one a mild recurrence, and one who had used betamethasone cream again, a severe recurrence.

It is suggested that fluorinated steroid applications can alter banal skin inflammation of the face into perioral dermatitis and that such applications are contraindicated in the treatment of facial eruptions.

PERIORAL dermatitis, originally described in the United States under the title of light sensitive seborrhoeid (Frumess and Lewis, 1957) and later under its present name (Mihan and Ayres, 1964; Hjorth *et al.*, 1968), and also facial dermatitis of unknown cause (Kaufman, 1965), remains something of a mystery. My reason for discussing it is twofold. Firstly, a remark in the paper by Hjorth *et al.* that studies of regional incidence of perioral dermatitis would seem to offer the best hope of obtaining a clue to the factors which induce it. Secondly, to report the results of treatment and follow-up of a number of treated cases.

DIAGNOSIS AND INCIDENCE

The diagnosis is simple because the features are so distinctive. The eruption becomes symmetrical, although it may start on one side of the chin or lip. It affects particularly the chin and the naso-labial folds, and may leave a clear zone round the lips (Fig. 1). The eruption, particularly if it has been treated with fluorinated steroids, can extend to the cheeks and forehead; but, unlike rosacea, there are no eye complications. The rash consists of firm erythematous papules, papulo-vesicles and papulo-pustules. In some cases, when scales form, resemblance to contact dermatitis is close. There are very wide variations in severity. Fig. 2 is that of a girl who started with mild acne vulgaris but had used betamethazone ointment for 6 months, and had a very unsightly eruption.

Seventy-three patients with perioral dermatitis have been seen in the last 2 years in our department. Whilst examples seen in the past have been diagnosed as seborrhoeic eczema or juvenile rosacea, there has been a very striking increase in the numbers which cannot be attributed to better recognition; unlike the cases described by Frumess *et al.*, patients have been seen all the year round, and exposure to sunlight does not appear to be an important factor.

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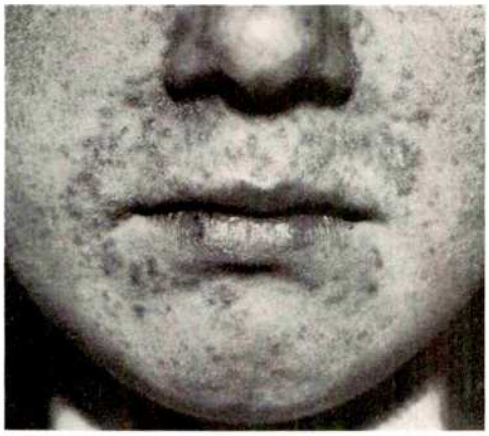

FIG. 1.—Case 1. A girl aged 16 who had used betamethasone valerate ointment for 6 months.

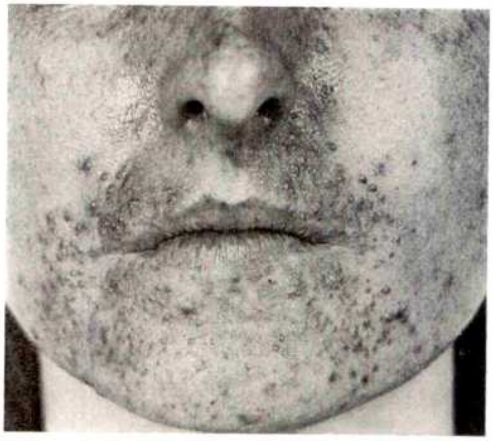

FIG. 2.—Case 2. Extensive lesions of perioral dermatitis.

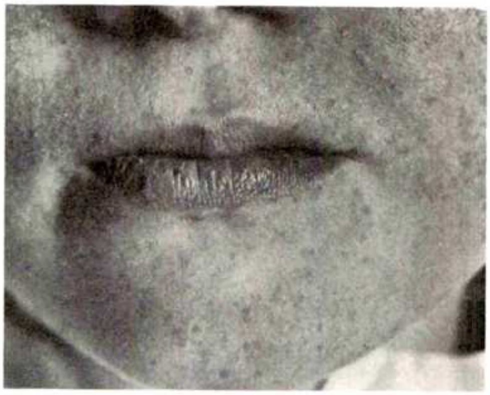

FIG. 3.—Case 1 after treatment for 1 month.

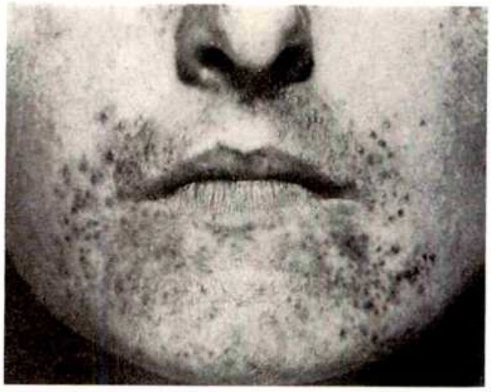

FIG. 4.—Case 2 after treatment for 1 month.

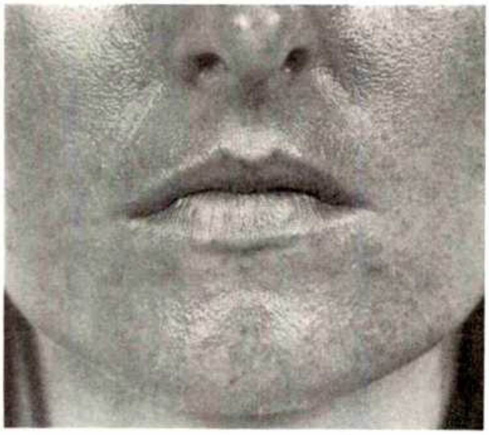

FIG. 5.—Case 2 after treatment for 5 months.

After discovery that rosacea could be aggravated by the application of topical fluorinated steroids in 1969 (Sneddon, 1969a), and the curious pustular rebound which occurred in many of these cases when steroid was stopped, a suggestion was made that a similar aetiology might be acting in perioral dermatitis (Sneddon, 1969b, 1970). Since that time particular attention has been paid to the history of medicaments that have been applied and to the actual mode of onset of the eruption in our patients. A common factor is that they have used fluorinated steroids with some temporary improvement. However, if the treatment is stopped, the condition recurs and is worse than before, and they are then forced to resume treatment; this is exactly the same story that one heard in fluorinated steroid-treated rosacea. Some of our patients have been applying fluorinated steroid twice or three times a day for several years. In many cases the topical steroids have not been prescribed by a doctor, but some quite trivial skin lesion, such as herpes simplex or a dry rough patch from chapping in cold weather, has been treated with steroid cream on the recommendation of friends and relatives that this was a universal panacea. In all but 2 of our patients with perioral dermatitis fluorinated steroids have been used.

It is my belief that topical steroids can aggravate, and in some cases induce, the syndrome, and this concept is supported by Baran and Civatte (1971) and Weber (1972).

Perhaps the most convincing evidence of the relationship of steroids to perioral dermatitis is the family of 3 who all presented suffering from the complaint. The mother, a pharmacist, had used betamethazone cream on her hands for a year for the treatment of eczema; later, when her face became red, she used it on her face. It is possible that she had actually contaminated her face from the ointment on her hands. Her husband then developed a rash on his face; and, since they shared a double bed, it is possible that steroid ointment from his wife's face was transferred to his. Finally, the child, who had a small area of chapping on the chin, was treated with topical betamethazone. Thus the whole family had been using betamethazone for some 6 months when they presented at the clinic. All recovered when hydrocortisone cream was substituted and oral tetracycline given to the adults.

That perioral dermatitis can occur in the very young has been pointed out by Verbov and Abell (1968), and I have recently seen an infant aged 10 weeks whose milk spots had been treated by an anxious parent with fluocinolone ointment for 9 weeks and who showed typical perioral dermatitis.

TABLE I

		Finley	*Hjorth et al.*
Present series	73 patients	43 patients	40 patients
Males	5	4	2
Females	68	30	38
Average age	28	30	29·6
Treated with topical steroids	71	Not known	Not known

Table I shows the figures for perioral dermatitis seen in Sheffield in the last 2 years and compares them with those already published by Hjorth *et al.* (1968) and Finley (1969).

The present series consisted of 73 patients of whom 5 were men and 68 women;

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the average age was 28. Of the 73, 71 had used fluorinated topical steroids. In Finley's series and that of Hjorth *et al.* the age group and distribution of male and female were the same, but no reference was made to the use of steroids.

It is a predominantly female complaint, and although there are some characteristic perioral cases of dermatitis in men, more usually fluorinated steroids produce only telangiectasia, with no papular and pustular elements.

We have not carried out histological studies on our patients, but this has been done on 26 such patients by Marks and Black (1971). They came to the conclusion that the histological changes were not dramatic and did not appear to be rosacea or simple eczema. There was no evidence of bacterial invasion or colonization with *Demodex.* The changes in the follicular epidermis were the most marked, and suggested that the disorder might be provoked by an external irritant of some kind.

We have carried out patch tests to the steroids and steroid bases in our patients and have demonstrated no evidence of contact sensitivity. Bacteriological and mycological studies of the pustules have not been helpful.

RESULTS OF TREATMENT

With regard to treatment, cessation of the fluorinated steroids was usually followed by aggravation of the eruption, which lasted some 8 to 10 days. Substitution of 1% hydrocortisone cream was occasionally sufficient to control the condition, but often did not. In the majority oxytetracycline was required in a dose of 250 mg twice daily. One important fact noted was that, when the patient had been given oxytetracycline and maintained on topical fluorinated steroids, the tetracycline did not appear to be of much benefit. This has also been noted by Weber. Thus patients have to be weaned off the topical steroid cream before tetracycline can control the eruption. The results of treatment have been most successful, more so than one would expect in rosacea, and virtually all have recovered on this regime.

Fig. 3 shows the great improvement in the patient shown in Fig. 1 after one month's treatment with hydrocortisone lotion and oxytetracycline 250 mg twice daily. Fig. 4 shows Case 2 after one month's treatment and Fig. 5 after 5 months' treatment. Complete recovery has been the general rule in patients who have managed to stop using the strong steroid applications.

TABLE II

Patients circulated	20	11 remained clear without further treatment
Replies	13	1 had slight recurrence controlled with local hydrocortisone
Males	1	
Females	12	1 had severe recurrence

A follow-up has been carried out on those patients who had undoubted perioral dermatitis necessitating treatment with oxytetracycline by mouth. Twenty of these patients were circularized 6 to 14 months after they had ceased all treatment. Of the 20, we received 13 useful replies. Of the 13 one was a man, 12 were women. Eleven had remained clear without any further treatment. One had had a slight recurrence which had been controlled by the use of hydrocortisone cream, and one had had a severe recurrence, but had resumed the use of betamethazone ointment. She was asked to attend the Department, and her condition was as severe as ever. She was once more persuaded not to use betamethazone and given oxytetracycline; the condition cleared and has remained clear for several more months.

DISCUSSION

It is still difficult to explain why powerful steroids can produce perioral dermatitis. Is it the fluorinated nature of the substance acting on a skin that is already inflamed, or is it merely its power to penetrate and damage the collagen? Further work being done in our department, using a powerful non-fluorinated steroid, may throw a little more light on this subject.

However, I feel certain that the moral should be that trivial banal skin inflammation of the face should not be treated with fluorinated steroids lest it be converted into perioral dermatitis.

I am grateful to my colleague Ronald Church for allowing me to include his patients in this review, and to Miss Wendy Spence for the illustrations.

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ULTRASTRUCTURAL FEATURES OF THE INVASIVE PHASE OF *CANDIDA ALBICANS*

R. A. CAWSON AND K. C. RAJASINGHAM

Summary.—Electron microscopy of oral epithelial plaque from patients with candidiasis confirms that the growth of candidas is intracellular and that the fine structure of the epithelial cytoplasm shows minimal change. The invading hyphae show well defined organelles, including a denticulate cytoplasmic membrane, nuclei, cristate mitochondria, rough and smooth endoplasmic reticulum, polyribosomes and storage granules. The fine double layer formation of the intra-cytoplasmic membrane structures is clearly demonstrable when using osmium tetroxide as fixative.

In both acute and chronic candidiasis (thrush, mucocutaneous candidiasis and candida leukoplakia) invasion of epithelium by hyphae of *Candida* is the prominent feature (Fig. 1). Ultrastructural studies by Montes and Wilborn (1968) have produced the unexpected and important finding that, unlike bacteria, *Candida albicans* in these conditions is an intracellular parasite penetrating the cytoplasm of the epithelial cell and presumably becoming dependent to some extent upon the latter for nutrition. However, studies upon the detailed ultrastructure of candidas have concentrated largely upon the readily cultured yeast form, and little information (apart from the findings of Montes and Wilborn, 1968) is available about the fine structure of the hyphae when growing within human epithelial cells.

MATERIALS AND METHODS

Specimens were taken from oral lesions of patients with either acute or chronic candida infections. In most cases the white plaque was detached by firm scraping with the short edge of a glass microscope slide, and the material transferred immediately into fixative. The fixative used was Palade's osmium tetroxide, buffered with veronal acetate to pH 7·4 at approximately 0°C for 2 hours. The material was dehydrated in graded ethyl alcohols, cleared in propylene oxide and embedded in hard grade Taab resin. Preliminary screening sections were cut at 2 μm on an L.K.B. Pyramitome, and stained with Paragon* for light microscopy.

Parts of the blocks thus selected and trimmed on the Pyramitome were then cut on an L.K.B. ultratome at 60 to 90 nm. The sections were mounted on cleaned, uncoated copper grids and stained in uranyl acetate and lead citrate. The grids were examined and photographed, using either an R.C.A. EM 3 or a Phillips EM 200 electronmicroscope.

Specimens from each patient were also cultured and identified by chlamydospore formation on cornmeal agar and by germ tube production (Taschdjian *et al.*, 1960) as *Candida albicans*.

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* Paragon multiple stain, PS 1301, C and C Paragon Co., Inc. 190 Willow Avenue, Bronx, New York 10454.

RESULTS

Relationship of hyphe to the epithelial cell.—The finding of Montes and Wilborn (1968) that the growth of *Candida* hyphae in human disease is intracellular is confirmed. It will be seen from the illustrations that, in all cases, the hyphae are surrounded by the tono-filaments of the epithelial cell. In some cases the hyphae are close to, or cross, the intercellular junctions. It did not appear, however, that there was any significant alteration in the arrangement of the tono-filaments or any other change in the organization of the epithelial cytoplasm in the vicinity of the invading hyphae.

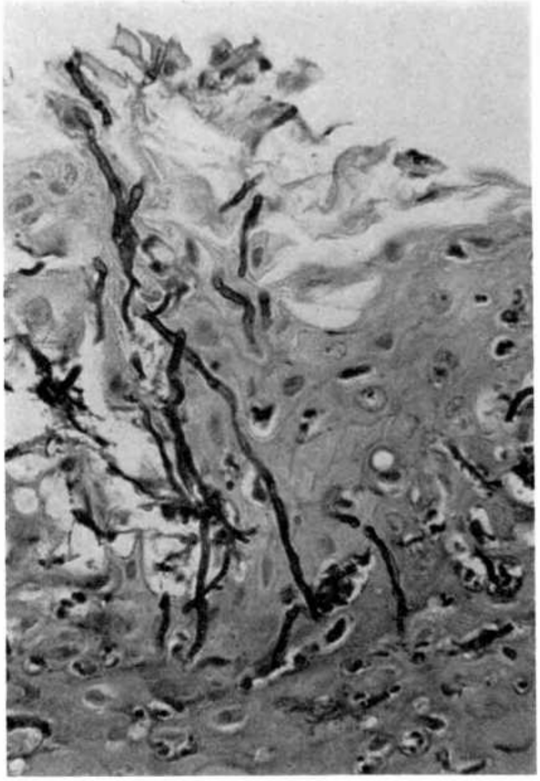

FIG. 1.—Epithelial plaque of chronic oral candidiasis. Hyphae are growing downwards in more or less straight lines and without respect for epithelial cell boundaries (H. and E. ×400).

Cell wall.—Using osmium tetroxide as fixative, the cell wall appears as an almost homogeneous structure of moderate electron density, but with a dense peripheral margin and a finer, dense inner surface which is distinct from the cell membrane. No fibrillar or other structural feature was evident in the substance of the cell wall itself. The cell wall in these preparations is estimated to be between 150 and 200 nm thick; this corresponds closely with Tsukahara and Sato's (1964) estimate for the thickness of the blastospore wall.

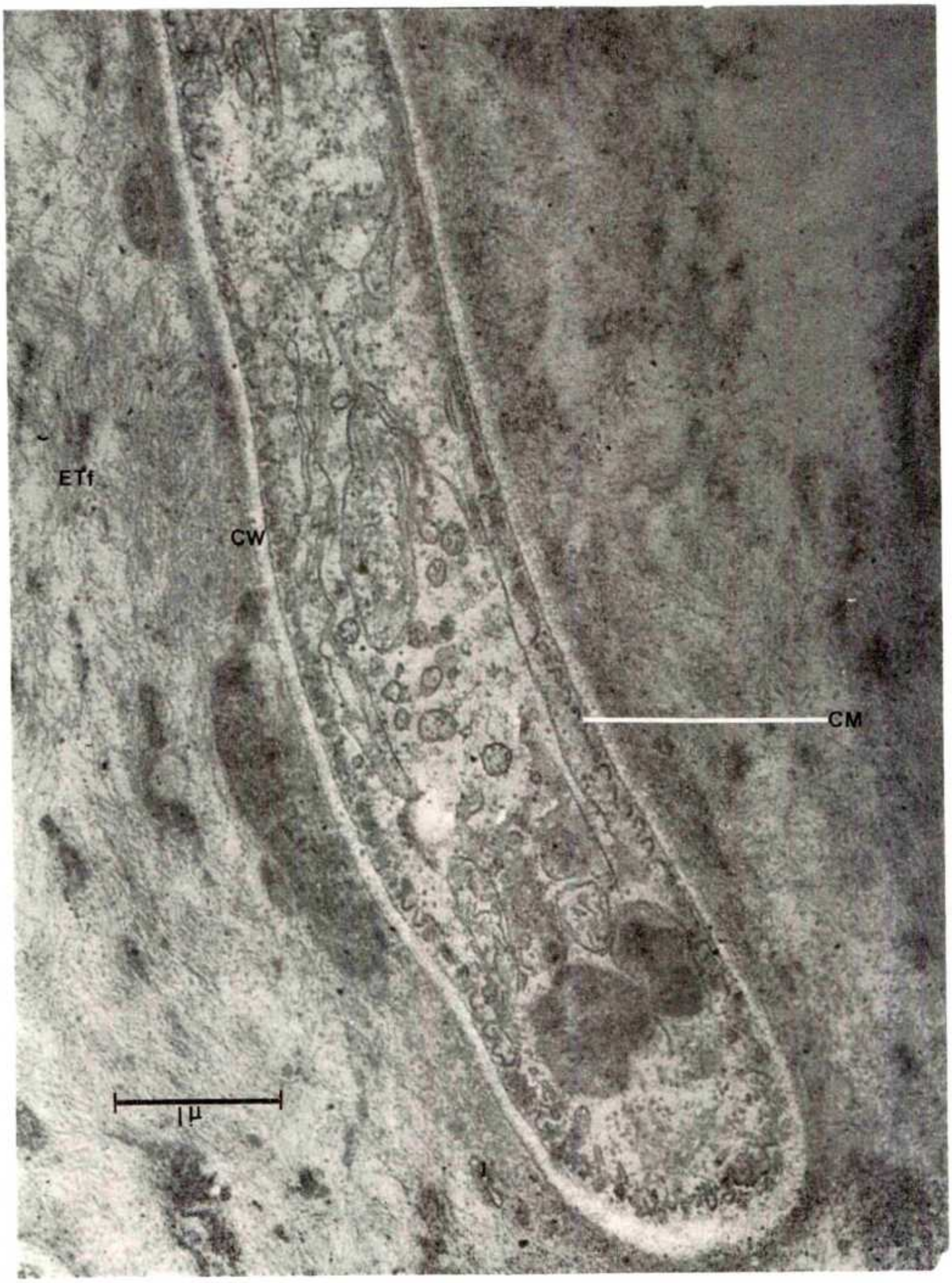

FIG. 2.—Electron micrograph of a longitudinal section of a *Candida* hypha within the cytoplasm of an epithelial cell. The tono-filaments (ETf) of the latter surround the hyphal cell wall (CW). The denticulate cytoplasmic membrane (CM) of the hypha is apparent, but the organelles are not easily identifiable in this section (× 27,000 approximately).

Cytoplasmic membrane.—This membrane is electron dense and, although described as undulant by Tsukahara and Sato (1964), it has in fact the same appearance in both transverse and longitudinal sections (Fig. 2). The cell membrane must therefore be micro-villous or denticulate in form. Under higher power (Fig. 3) it is evident that this membrane is double-layered. It can be seen also that there are discontinuities in the membrane which is also in places elabor-

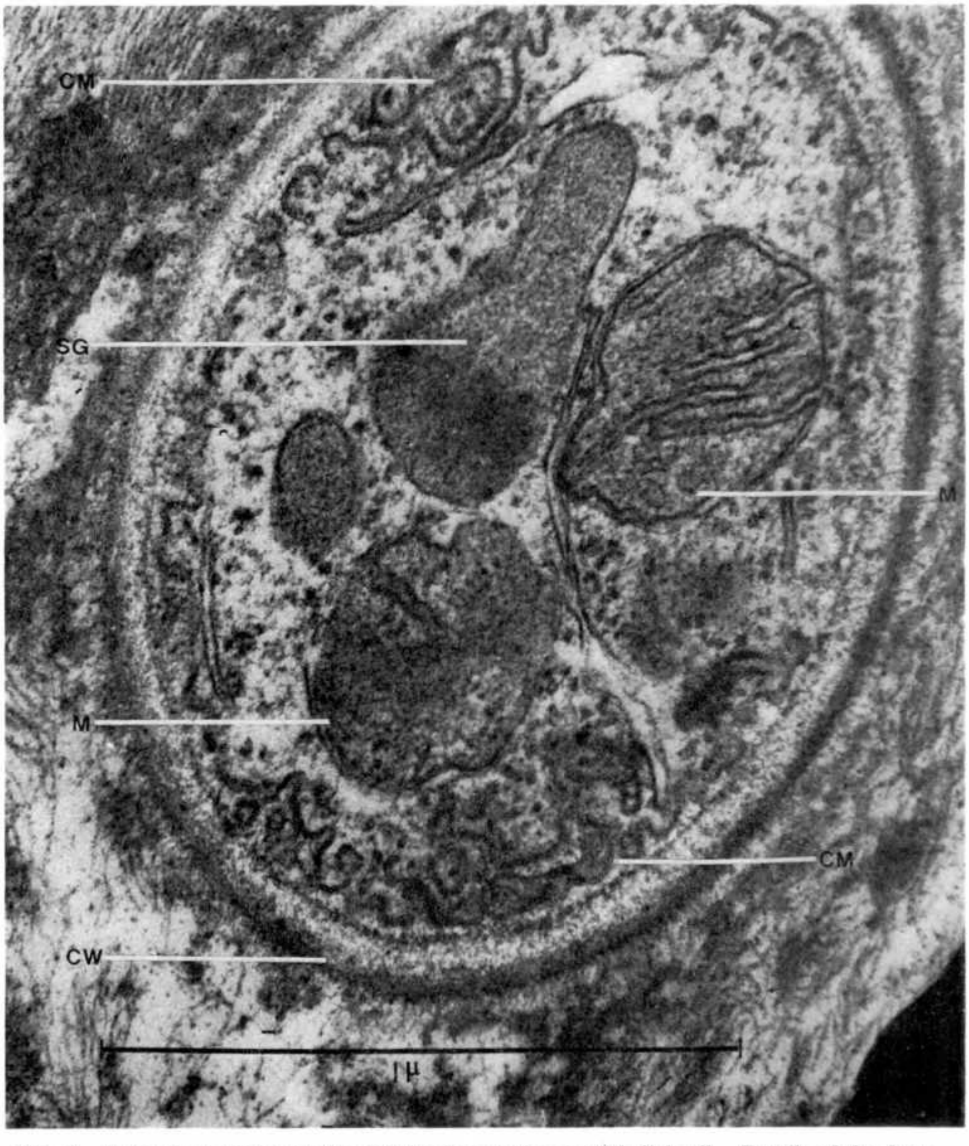

FIG. 3.—Transverse section of *Candida* hypha within an epithelial cell. Details of the dense inner and outer layers of the cell wall (CW), the double layer structure of the cytoplasmic membrane (especially clear at the points marked CM), mitochondria with cristae (M) and storage granules (SG) are among the features that can be seen (× 103,000 approximately).

ately convoluted, either due to oblique cutting of invaginations or because it joins other membrane structures.

Nucleus.—A rounded structure showing the characteristic pored, double membrane and containing dense granular material can be seen (Fig. 4). This illustration shows also that there is wider separation of the layers forming the nuclear membrane than of the cell membrane. The nucleus, however, appears unexpectedly large, and almost fills the cell in these sections. No nucleoli have been seen.

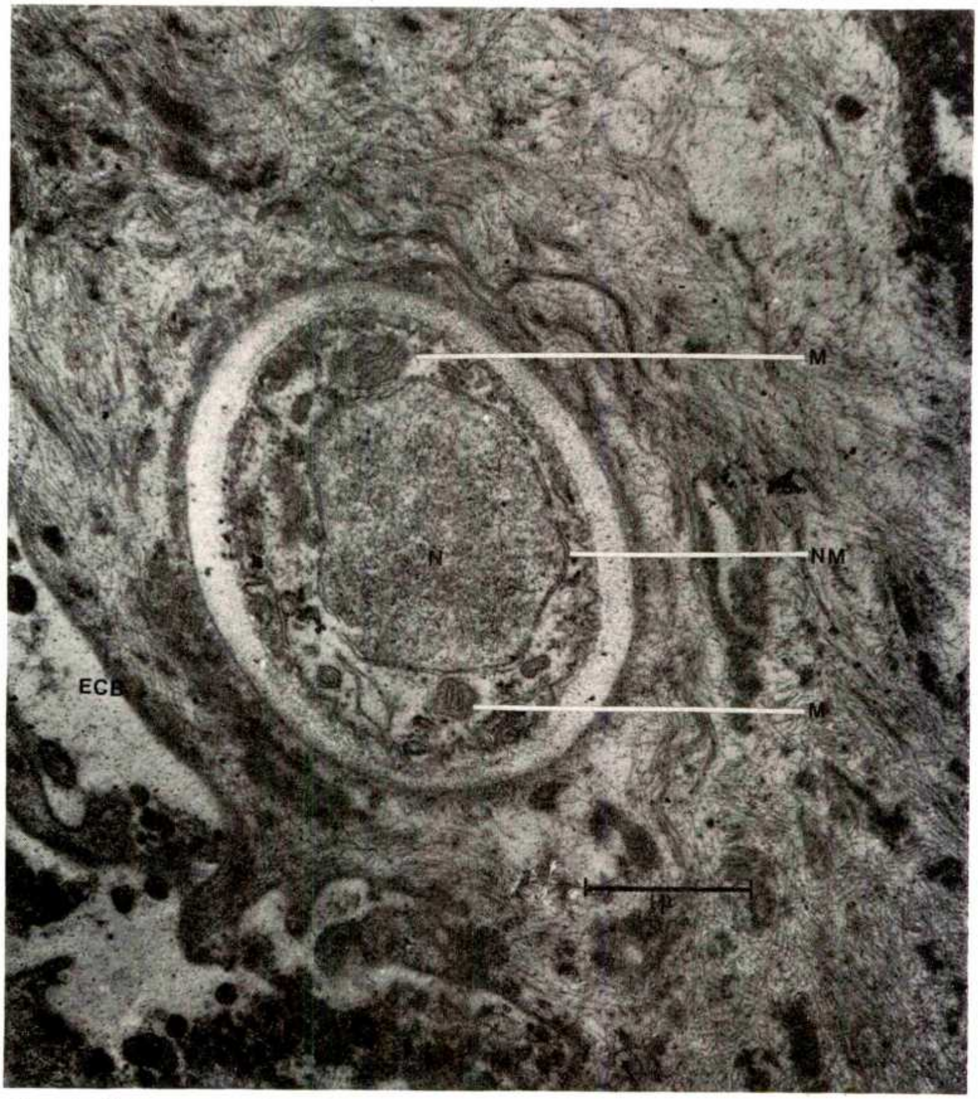

FIG. 4.—Transverse section of hypha, showing nucleus (N) with double membrane (NM) interrupted by pores and small cristate mitochondria (M). The boundary of the epithelial cell (ECB) in which the hypha is growing is towards the bottom left of the picture (× 27,000 approximately).

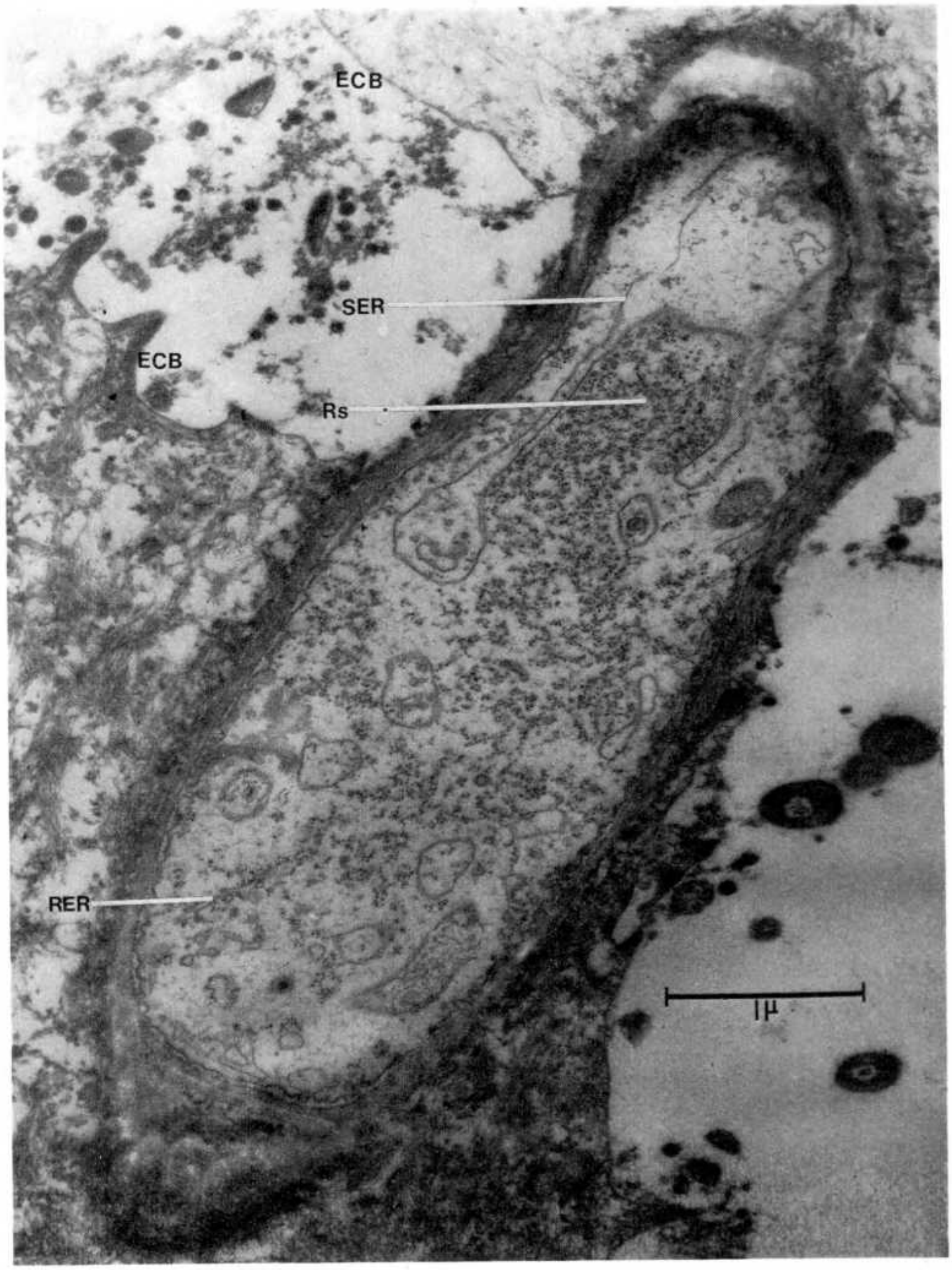

FIG. 5.—An oblique section of a hypha passing across the boundary between (ECB), partially within 2 epithelial cells. Prominent features are the polyribosomes (Rs) and endoplasmic reticulum (SER). A short length of rough endoplasmic reticulum (RER) is apparent near the lower left hand end of the hypha (× 25,000 approximately).

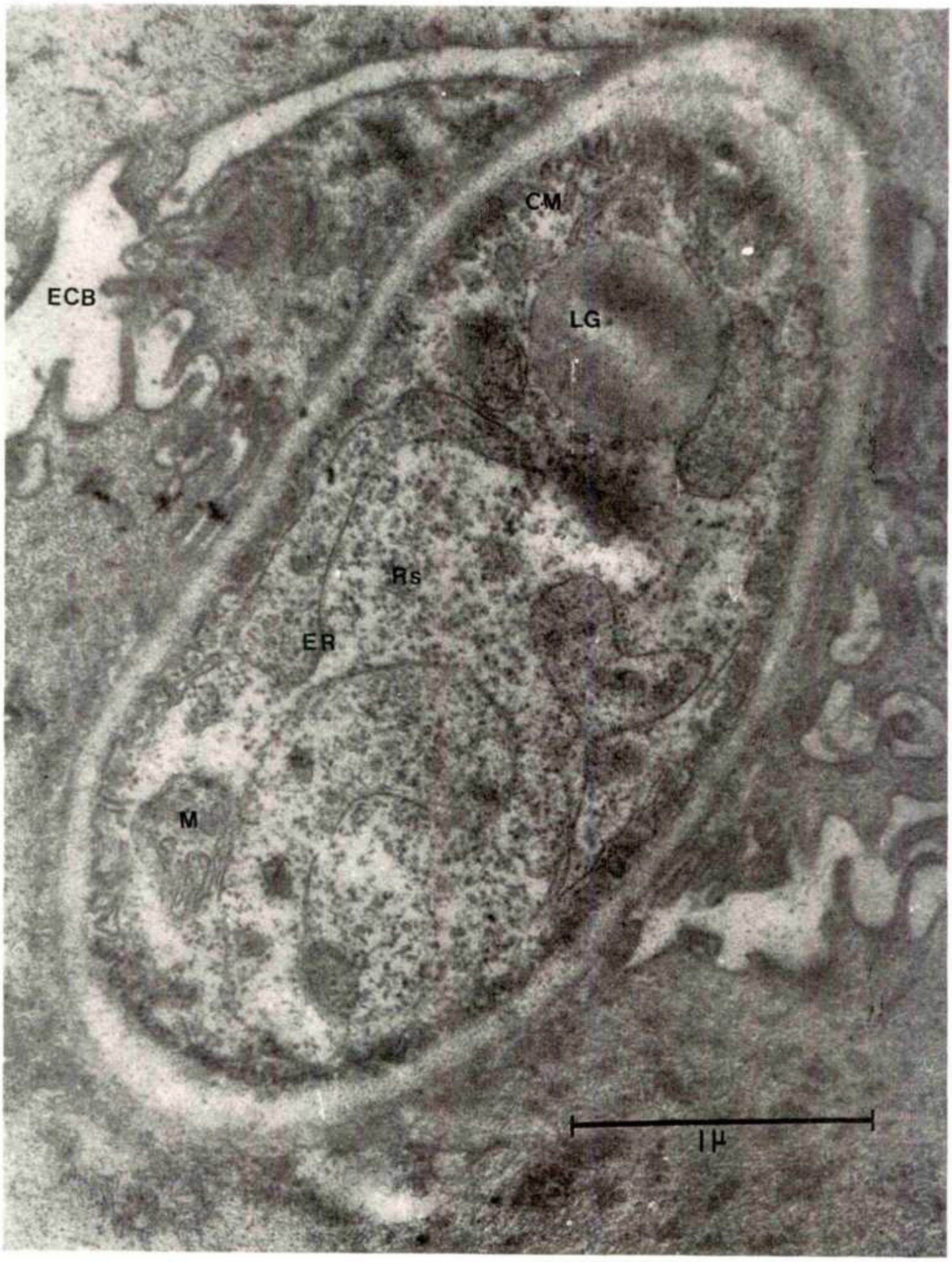

FIG. 6.—Oblique section of a hypha crossing epithelial cell boundaries (ECB) and showing a lipid granule (LG), endoplasmic reticulum (ER), polyribosomes (Rs) and a mitochondrion (M) (× 45,000 approximately).

Mitochondria.—These can be clearly seen in many sections, and show well-defined, parallel cristae, although these vary widely in number in individual mitochondria. The mitochondria also appear to differ widely in size, as comparison of Fig. 3 and 4 shows. It is noticeable that the cristate structure is apparent even in the most minute of these mitochondria. It seems possible that some of the other organelles of similar size and form are less highly developed mitochondria lacking cristae.

Endoplasmic reticulum and ribosomes.—Double-layered internal membranes are present in virtually all sections, and are prominent and extensive in some. The shorter lengths of this membrane might possibly be collapsed vacuoles, but are probably part of a larger structure. The ramification of this internal membrane, although irregular, is so extensive in the longitudinal and in some transverse sections (Fig. 2, 5, 6) that it clearly forms part of an organized system.

The probability is that this membrane, although mostly smooth, is endoplasmic reticulum. This is made more likely by the fact that, in Fig. 5, ribosomes can be seen attached to a portion of this membrane system, giving the appearances of rough endoplasmic reticulum. Elsewhere, organelles of the characteristic size and form of polyribosomes are loosely arranged near the centre of the cell, but not in immediate proximity to the endoplasmic reticulum.

Storage granules and lipid bodies.—A number of areas are seen with what seems to consist of a single limiting membrane and dense, granular contents. These may be somewhat unimaginatively termed " storage granules ", and resemble those described by Agar and Douglas (1957) in *Saccharomyces cerevisiae*. It will be seen, however, in Fig. 3 that there is a single, well-defined, cristate mitochondrion with an almost complete double-layered limiting membrane. Nearby is another mitochondrion, with denser granular contents, a single well-defined crista (but much less distinct cristae), and an outer membrane of which only a small part is double layered. Finally, in close proximity to both, is a " storage granule " which is, however, of similar size and with granular contents of similar density to the mitochondria, and which could be regarded as representing a further stage in the loss of identifying detail.

DISCUSSION

All the structures described above have been demonstrated in the yeast form (blastospores) of *C. albicans* or *S. cerevisiae* by previous workers such as Palade and Porter (1954), Agar and Douglas (1957), Gale (1963), Bakerspiegel (1964) and Tsukahara and Sato (1964). In these preparations of yeasts or blastospores, however, the details of the membranous organelles and of the membranes themselves tend to be obscured by electron-dense, granular material. The clear definition of the organelles in the invading hyphae shown here may be due to one or several of the following factors. First, different fixative agents, such as glutaraldehyde or potassium permanganate, have been used by most of the previous workers, and this almost certainly will have affected the picture, although to what extent is by no means clear. Secondly, the metabolism of the organism is likely to change as it colonizes the epithelial cell, and it is possible that the functional organelles in full activity may become more clearly defined or that the widespread granular material shown in previous reports is used up or undergoes some other change. Thirdly, it may be simply that, as the hypha elongates, the organelles are dispersed along this structure instead of being crowded together in the confines of the blastospore. Of course, more organelles are likely to develop as the hypha extends, but possibly not in such numbers in relation to the greater volume available.

The study of Montes and Wilborn (1968) is most closely comparable to the present work in that it is concerned with the invading hyphae. A different method of fixation was used, however (glutaraldehyde and acrolein, with postfixing in osmium tetroxide), and differences in the ultra-structural features are apparent. Thus, in Montes and Wilborn's (1968) preparations, (1) the cell wall is of very low electron density; (2) the cytoplasm is virtually filled either with electron-dense material or with large clear vacuoles; (3) large myelin figures and other multilayered structures are apparent where the cytoplasm is most electron-dense.

There still remains considerable scope for further investigation of the different phases of growth of *C. albicans* in relation to the various methods of preparation. Full evaluation of the ultrastructure of this important organism therefore depends to some extent upon the time-consuming and painstaking work necessary to determine and consistently reproduce the " normal " features under each condition of growth.

We would like to express our gratitude for the invaluable help given by Dr W. Bannister of Guy's Hospital Medical School and Dr P. Walker of the Wellcome Institute, and to the Guild of Expatients and Friends of Guy's Hospital whose generous grant provided for some of the apparatus.

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THE "WHEAT-STUBBLE SARCOID GRANULOMA": A NEW EPITHELIOID GRANULOMA OF THE SKIN

J. CORTEZ PIMENTEL

Summary.—The introduction into the skin of chemical substances or organic matter can produce granulomata of sarcoid type. A new granuloma of this type related to wounds produced by the stubble of wheat is described. The diagnosis of this lesion is easy, taking into consideration its localization in the lower limbs, the wounds caused by stubble, and the identification of intracellular plant material in the granuloma. A host factor of probable immunological nature is suggested. There is a similarity between these sarcoid granulomata of the skin and some epithelioid granulomatous lesions of the lung due to the inhalation of chemical substances and organic matter, known as " extrinsic allergic alveolitis ".

THE inoculation into the skin of chemical substances and of organic matter of animal or vegetable origin can produce epithelioid, sarcoid-type granulomata. Such lesions have been reported as caused by silica (Sommerville and Milne, 1950; Epstein, 1955), by talcum (Fienberg, 1937), by beryllium (Dutra, 1949), by the sulphonamides (Duperrat, 1952), by zirconium (Rubin *et al.*, 1956; Shelley and Hurley, 1958) and by cactus (Winer and Zeilenga, 1955) and sea-urchin (Kinmont, 1965) spines.

This paper describes a sarcoidal granuloma due to particles of gramineae inoculated into the skin by wheat stubble left after reaping. We have also attempted to explain the resemblance between these sarcoidal granulomata of the skin and epithelioid granulomata of the lung due to the inhalation of chemical or organic matter, known as " extrinsic allergic alveolitis " (" farmer's lung" , Pepys *et al.*, 1964; Seal *et al.*, 1968; " bird fancier's lung ", Nash *et al.*, 1967; " suberosis ", Cancella, 1955; " furrier's lung ", Pimentel, 1970; and the " lung of worker's in the plastic material industry ", Gomes, 1971).

CASE REPORT

A white woman aged 51 was admitted to the Surgical Department of Santa Maria Hospital in March 1959. Approximately 2½ years earlier she noted pain at rest on the inside of the right ankle. This was accompanied by oedema and slight redness, followed some weeks later by a purplish-red, flat, umbilicated nodule about 1·5 cm in diameter. Many local treatments were undertaken and various antibiotics were administered, but there was no improvement. Some months later, " because of a knock with pruning scissors ", the lesion became worse and ulcerated. A presumptive diagnosis of tuberculosis was made and surgery undertaken. Histological examination of the excised tissue showed that the dermis was infiltrated by a predominantly sarcoid type epithelioid granuloma, made up of discrete and compact epithelioid tubercles, surrounded by

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lymphocytes and many Langhans and foreign body type giant-cells (Fig. 1, 2). A few small necrotic foci were seen, but no histiocytes in palisade arrangement were noted; some of the giant cells contained inclusion bodies (Fig. 2), none of which stained for calcium (von Kossa's stain). Ziehl-Neelsen's stain showed no acid-fast rods, and Gomoris' and Unna-Pappenheim's techniques revealed no fungi or leishmania.

An x-ray examination of the ankle showed no bone lesions. Routine laboratory tests, such as E.S.R., blood count and serum protein electrophoresis, were within normal limits. Culture of the residual tissue also showed no tubercle bacilli. However, the patient was treated with streptomycin and isoniazid, and the lesions healed.

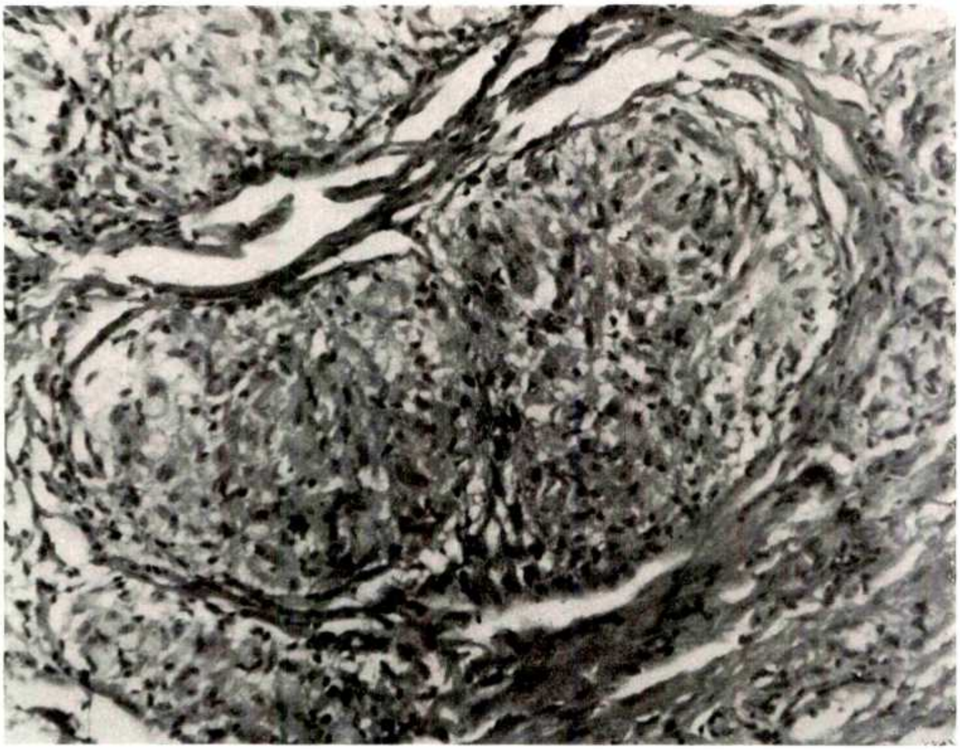


FIG. 1.—Sarcoid-type granuloma infiltrating the dermis of the medial aspect of the right ankle (H. and E. × 60).

The patient was re-examined in June 1971 and reported that 8 years previously, 5 years after the lesions on her ankle, she had had a similar lesion on the lateral aspect of the lower third of the right leg, which had also been treated surgically (Dr Rui de Lima, Sanatório D. Carlos I). In their files we found the description of a nodular, purplish-red skin lesion, approximately 1·5 cm in diameter, located on an oedematous and congested area of the skin. It was slightly painful, and there were no signs of suppuration. Histological examination showed a sarcoid-type epithelioid granuloma similar to that seen on the right ankle. No tubercle bacilli could be demonstrated in direct smears or following culture or guinea-pig inoculation. The case was again considered to be tuberculous, and the patient was treated for some months with anti-tuberculous drugs.

The patient had worked on a farm since the age of 20. The lesion on the right leg had developed approximately one month after she had pricked herself on wheat stubble.

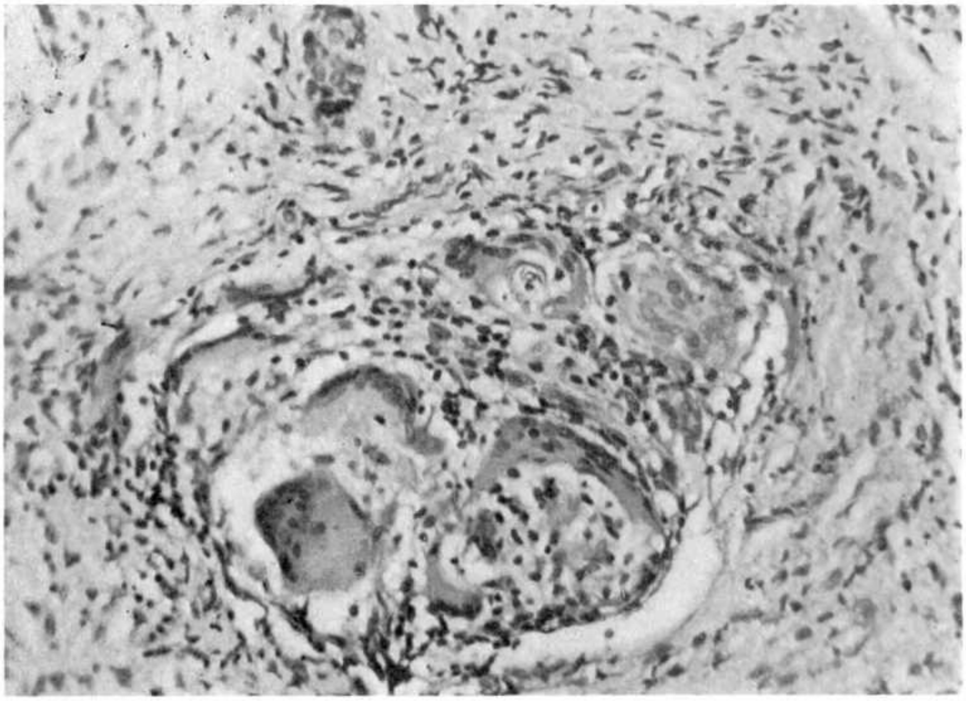
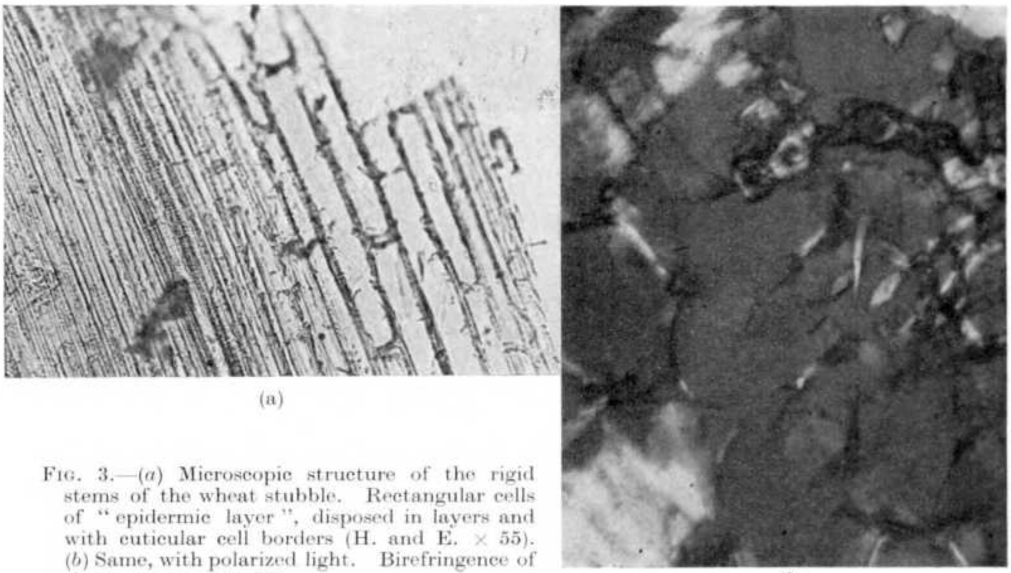


FIG. 2.—Another view of the same granuloma. Epithelioid follicle, bounded by lymphocytic infiltrates and numerous giant cells both Langhans and foreign body type. Some of the giant cells contain "inclusion bodies". (H. and E. × 75).



(a)

(b)

FIG. 3.—(*a*) Microscopic structure of the rigid stems of the wheat stubble. Rectangular cells of "epidermic layer", disposed in layers and with cuticular cell borders (H. and E. × 55). (*b*) Same, with polarized light. Birefringence of these structures (× 75).

For many years she had frequently pricked herself on the stubble, but the wounds had healed rapidly. After the latest operation she has worn high rubber boots to avoid further injury.

METHODS

Histological examination of the rigid stem of wheat stubble.—This study was carried out in fresh sections mounted on slides, using as vehicle glycerine and Apathy's syrup. Some sections were stained with carmine-green iodine (Costa and Chaves, 1943) and, after embedding in paraffin, with H. and E., PAS, zinc chloride–iodine solution and iodine–potassium iodide solution (Lugol's solution) for cellulose (Lillie, 1965; Koch, 1963), ruthenium red (Lillie, 1965; Koch, 1963), and carmine-green iodine for staining lignin (Costa and Chaves, 1943). Both standard and polarization microscopes were used.

Histological examination of the skin lesions obtained at both biopsies.—Fresh sections fixed in neutral 10% formalin and stained with H. and E., PAS, and with zinc chloride–iodine solution and Lugol's solution for cellulose, and ruthenium red and carmine-green iodine for lignin. On sections embedded in paraffin and fixed in neutral 10% formalin we carried out the following staining techniques: H. and

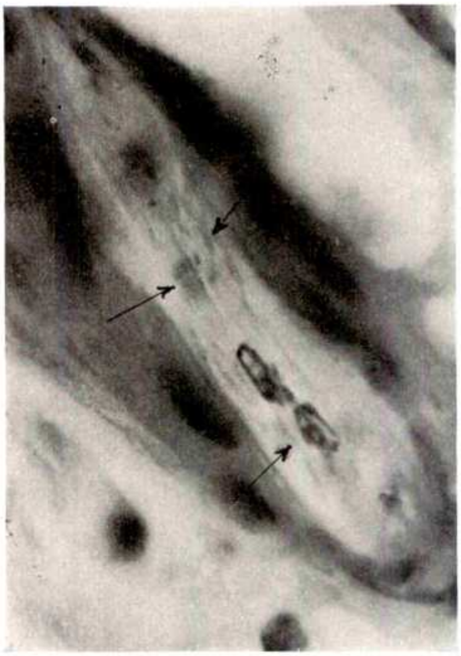


FIG. 4.—Detail of a foreign body giant cell, with "inclusion bodies" in the skin granuloma of lower third of the leg. Note that "inclusion bodies" are very similar to the cellular structure of the wheat stem (Fig. 3a) (H. and E. × 600).

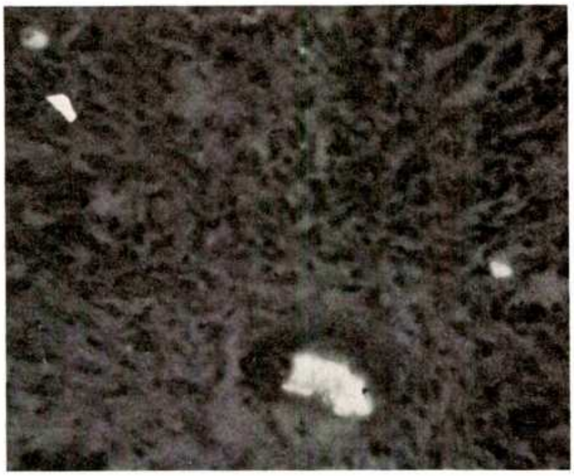


FIG. 5.—Granuloma of the medial aspect of the right ankle, seen with partially crossed Nicol prisms. Birefringence of the "inclusion bodies" (H. and E. × 75).

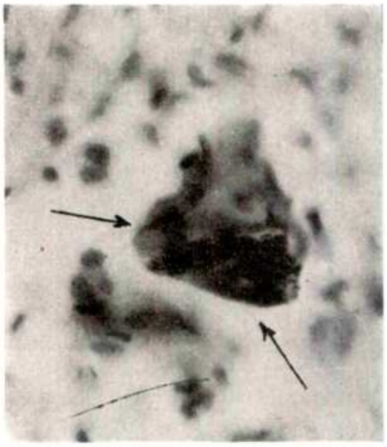


FIG. 6.—"Inclusion body" stained with Lugol's solution for cellulose. The black colour (blue in sections) is the usual reaction of the cellulose (× 80).

E., Wilder for reticulin fibres. Weigert's resorcin-fucsin for elastic fibres, Van Gieson, PAS, Ziehl-Neelsen, von Kossa, Unna-Pappenheim, Gomori for fungi, zinc chloride–iodine solution, Lugol's solution, for cellulose, and ruthenium red and carmine-green iodine for lignin.

RESULTS

The histology of the rigid cut end of wheat stubble showed that it was composed predominantly of layers of rectangular cells with cuticular cell borders (Fig. 3a). These cells gave positive stains for cellulose (zinc chloride–iodine and Lugol positive) but not for lignin (carmine-green and ruthenium red negative). They were also birefringent (Fig. 3b) and PAS positive.

Microscopy of the skin lesions, stained with H. and E., showed that some foreign body giant-cells contained vacuoles with rectangular, fasciculated "inclusion bodies", (Fig. 4), similar in structure to wheat stem (morphology, birefringent—Fig. 5—and affinity to PAS). Cellulose staining reactions suggested their vegetable nature (Fig. 6). Moreover, epithelioid cells of the granuloma sometimes contained fine particles with similar histochemical and histophysical characteristics.

DISCUSSION

This female farm worker aged 51 had a sarcoid type granulomatous epithelioid lesion of the medial aspect of the right ankle, in which no tubercle bacilli could be demonstrated. Years later a similar lesion on the right leg was confirmed histologically. She reported that this latter lesion had developed after she had pricked her leg on wheat stubble. The inclusion bodies found in the foreign body giant-cells and, sometimes, in the epithelioid cells of the granuloma, were considered to be small particles of the epidermal layer of the stem of wheat stubble. We tried to confirm this hypothesis by a comparative morphological study of wheat stems and of the lesions, and also by histochemical reactions for cellulose. Our findings suggest that this is a sarcoid granuloma of the skin analogous to those secondary to silica, talcum, beryllium, sulphonamides, zirconium and in wounds from cactus and sea-urchin spines.

The diagnosis of this new granuloma is easy, taking into consideration its localization in the lower limbs, especially the ankles and lower third of the leg, with a history of trauma from stubble, and the identification of intracellular plant material in the granuloma (Fig. 6).

This type of granuloma seems to have a low incidence in relation to the relative frequency with which rural workers and hunters are wounded by wheat stubble. This suggests that the development of this lesion requires an "individual", probably immunological factor. It may be possible to explain the time lag between the inoculation and the formation of the granuloma by a delayed sensitization mechanism of the type found in Kveim's reaction, and in beryllium (Curtis, 1951), zirconium (Shelley *et al.*, 1958) and sea-urchin (Kinmont, 1965) granulomata.

We believe there may also be a close relationship between these secondary granulomata of the skin and the epithelioid granulomatous conditions of inhalatory origin found in the lungs (Pepys *et al.*, 1964; Seal *et al.*, 1968; Nash *et al.*, 1967; Avila and Villar, 1968; Pimentel and Marques, 1969; Pimentel, 1970; Pimentel, 1971). These pulmonary diseases affect the lung tissue diffusely, often leading to irreversible respiratory insufficiency; on the other hand, the skin lesions are localized in character and their main hazard is misdiagnosis as tuberculosis, resulting in unnecessary anti-tuberculous therapy. Both the skin and lung lesions can be produced by chemical substances (Dutra, 1949; Pimentel and Marques, 1969; Gomes, 1971) and by organic matter of animal or vegetable origin (Nash *et al.*, 1967; Seal *et al.*, 1968; Avila and Villar, 1968; Pimentel, 1970; Pimentel, 1971). Their structure is usually that of an epithelioid granuloma, and the " aggressive agents " seem always to be present in the lesions (Dutra, 1948; Sommerville and Milne, 1950; Pimentel and Marques, 1969; Pimentel, 1970; Gomes, 1971), although they may be difficult to identify.

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ACID HYDROLASES IN THE HUMAN EPIDERMIS

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Summary.—α- and β-D-glucosidases, β-D-glucuronidase, α- and β-D-mannosidases, α- and β-D-galactosidases, β-D-fucosidase, α-L-fucosidase and β-D-N-acetylglucosaminidase activities were measured in the human epidermis using p-nitrophenol and 4-methylumbelliferone compounds as substrates. There was no appreciable difference in activities noted when both substrates were compared. Acid phosphatase was assayed with p-nitrophenol phosphate and α-naphthyl acid phosphate as substrates. Acid phosphatase seemed to be present, both free in the cytoplasm and in lysosomes. These 2 activities could not be differentiated by agarose electrophoresis (both fractions showed 2 anodally migrating bands).

Some of the biochemical properties of these acid hydrolases from the normal human epidermis are presented.

INBORN deficiencies of acid hydrolases which are responsible for the degradation of macromolecular carbohydrates cause many diseases. Symptoms result from the accumulation of these macromolecules in various tissues and organs.

These deficiencies include ceramide trihexosidase in Fabry's disease (Mapes *et al.*, 1970), β-galactosidase in Hurler's disease (Öckerman, 1968), Hunter's disease (Gerich, 1969), Landing disease (Dancremont and Kint, 1968); acid maltase in Type II glycogen storage disease (Leathwood and Ryman, 1970); β-N-acetylglucosaminidase in Tay-Sachs disease (Friedland *et al.*, 1970) and β-glucosidase in Gaucher's disease (Öckerman and Kohlin, 1968; Hultberg and Öckerman, 1970).

The activity of these enzymes is generally measured in hepatic or splenic tissue obtained by biopsy. The demonstration that the specific enzymes are also deficient in the skin in Hurler's, Hunter's, and Type II glycogen storage disease (Öckerman, 1968; Gerich, 1969; Leathwood and Ryman, 1970) opens the way to the use of the skin biopsy as a less hazardous and easier method of the rapid diagnosis of these conditions.

This communication will report several acid hydrolase activities in the normal human epidermis and will discuss some of the biochemical properties of the enzymes.

MATERIALS AND METHODS

Materials

4-Methylumbelliferyl (4-MU)-β-D-glucoside, 4-MU-β-D-glucuronide, 4-MU-β-D-mannoside, 4-MU-α-D-galactopyranoside, 4-MU-β-D-galactopyranoside and 4-MU-2-acetamide-2-deoxy-β-D-glucopyranoside were obtained from Koch-Light Laboratories,

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Ltd., Colnbrook, Bucks, England. They were stored desiccated at −4°C and 60 mmol/l solutions were freshly prepared in 0·2 mol/l acetate pH 4·5 buffer for the assays of relative activities. Sixty mmol/l solutions of each substrate in 0·2 mol/l acetate buffer of differing pH values was used for pH optima studies.

p-Nitrophenyl (PNP)-β-D-glucoside, PNP-α-D-glucoside, PNP-β-D-glucuronide, PNP-α-D-galactopyranoside, PNP-β-galactopyranoside, PNP-α-D-mannoside, PNP-β-D-mannoside, PNP-α-L-fucoside, PNP-β-D-fucoside, PNP-N-acetyl-β-D-glucosaminide, dicyclohexylammonium salt of PNP-phosphate and sodium α-naphthyl acid phosphate were purchased from Sigma Co., St Louis, Missouri. They were stored desiccated at −4°C and 60 mmol/l solutions were freshly prepared in 0·2 mol/l acetate, as in the case of 4-MU compounds. Sodium glucose-6-phosphate and sodium triphosphopyridine nucleotide were obtained from Calbiochem, San Diego, California and 100 mmol/l and 10 mmol/l solutions were made in redistilled water.

Agarose, special grade for electrophoresis, was obtained from Mann Research Lab., N.Y. Fast Blue RR (special) was obtained from Calbiochem, San Diego, California.

Methods

Preparation of the homogenates.—Keratome slices were obtained from the backs of volunteers with no apparent skin disease with a motor-driven Castroviejo Keratome, as described previously (Ohkawara *et al.*, 1972). The slices contain approximately 85–90% epidermis and 10–15% dermis. The slices were immediately frozen in liquid nitrogen and weighed while frozen; 5% homogenates were made with redistilled water in a glass homogenizer in the cold. These homogenates were used as the source of enzymes.

Assay procedures for the acid hydrolases.—Various acid hydrolases were assayed according to Bessey *et al.* (1946), with a slight modification. The reaction mixture consisted of 100 μl substrate, 100 μl of the enzyme preparation and 300 μl of redistilled water. The final volume of the reaction mixture was 500 μl. For the substrate and enzyme controls, 400 μl of redistilled water was used instead. The reaction tubes were placed in a 37°C water bath and incubated for 30 min for acid phosphatase and for acetyl-N-glucosaminidase, and for 60 min for the remainder of the enzymes. At the end of the incubation periods 2·5 ml of 0·1 mol/l glycine buffer, pH 10·5, was added to the PNP substrates, and 4·0 ml of the same buffer to the 4-MU and α-naphthol substrates to stop the reactions. The liberated p-nitrophenol was read at 400 nm in a Beckman spectrophotometer. α-Naphthol and 4-MU were read fluorometrically in a Farrand spectrophotofluorometer at 355 nm (excitation) and 450 nm (emission) for α-naphthol, and 370 nm (excitation) and 455 nm (emission) for 4-methylumbelliferone. Protein contents were determined according to Lowry *et al.* (1951).

Fractional centrifugation.—Five per cent homogenates of fresh tissues in 0·25 mol/l sucrose were used. The homogenates were centrifuged at 700 *g* for 20 min, and the supernatants obtained were further centrifuged at 50,000 *g* for 60 min; 50,000 *g* precipitates were suspended in a small volume (usually 200–300 μl) of the 0·25 mol/l sucrose and used as presumed lysosomal fractions. All of the centrifugations were carried out in a refrigerated International Centrifuge, temperature controlled at 4°C. Glucose-6-phosphate dehydrogenase was assayed as described previously (Halprin and Ohkawara, 1967).

$HgCl_2$ inhibition study.—The enzyme preparations were incubated for 20 min at room temperature with 3 mmol/l $HgCl_2$ (final concentration). After 20 min incubation, each substrate was added and the incubations extended another 60 min at 30°C. The reactions were stopped by adding 2·5 ml of 50 mmol/l glycine buffer, pH 10·5. Substrate and enzyme controls with 3 mmol/l $HgCl_2$ were also run.

Agarose electrophoresis for acid phosphatase.—Agarose 0·5% gel was prepared with 50 mmol/l barbital buffer, pH 8·7. Agarose solutions were brought to the boil, cooled for 2 min at room temperature; α-naphthyl acid phosphate was then added to make a

0·1% concentration. Electrophoretic slides were prepared by pouring the hot agarose on to 1 in × 3 in glass microscope slides with an appropriate slot former and allowing them to cool. Twenty μl of each fraction obtained from the fractional centrifugation containing 1% triton-X-100 was applied in each slot, and a horizontal electrophoresis was carried out for 180 min at 4°C at 100 volts DC. Fifty mmol/l barbital buffer, pH 8·6, was used as electrode buffer. The freshly made staining mixture consisted of 100 mg of fast blue RR salt, 100 mg of α-naphthyl acid phosphate and 0·001% $MgCl_2$ in 100 ml of 0·1 mol/l (final concentration) acetate buffer, pH 4·0. The gels were submerged in the staining solution for at least 3 hours at room temperature.

RESULTS AND DISCUSSION

1. *Activities of Various Acid Hydrolases in the Normal Human Epidermis*

The results are shown in Table I. Most of the enzyme activities were assayed using p-nitrophenol compounds as substrates. β-D-glucosidase, β-D-glucuronidase and β-D-galactosidase activities were compared, using both p-nitrophenol and 4-methylumbelliferone compounds as substrates. Although there was no appreciable difference in the relative activities, 4-methylumbelliferone compounds (fluorometric assay) offered much higher sensitivities. Acid phosphatase activities were compared, using p-nitrophenol phosphate and α-naphthyl acid phosphate (fluorometric assay) as substrates. The former gave approximately twice the activities obtained with the latter substrate. β-Galactosidase, β-N-acetylglucosaminidase and β-glucuronidase activities presented in Table I are considerably higher than those reported by Öckerman (1968) and Gerich (1969). This could be due to the different type of skin specimens analysed. Our keratome slices contain approximately 80–90% epidermis, while in their studies whole skin was used (Öckerman, 1968; Gerich, 1969). Although the normal enzyme activities cover a wide range, the activities in patients with congenital metabolic disease are only 10% to 30% of normal. It is, therefore, possible to detect these congenital disorders by using our keratome slices.

TABLE I.—*Acid Hydrolases Activity in the Normal Human Epidermis*

Enzyme	nm/min/mg protein	(Range)	Remarks
α-D-glucosidase	0·34 ± 0·22 (4)	(0·25 — 0·74)	PNP
β-D-glucosidase	0·46 ± 0·03 (3)	(0·36 — 0·58)	PNP
	0·65 ± 0·15 (2)	(0·50 — 0·80)	4-MU
β-D-glucuronidase	0·83 ± 0·22 (5)	(0·54 — 1·15)	PNP
	0·59 ± 0·16 (3)	(0·40 — 0·80)	4-MU
α-D-mannosidase	0·78 ± 0·20 (4)	(0·56 — 1·12)	PNP
β-D-mannosidase	0·95 ± 0·25 (4)	(0·51 — 1·12)	PNP
α-D-galactosidase	0·68 ± 0·24 (5)	(0·25 — 0·96)	PNP
β-D-galactosidase	0·59 ± 0·26 (4)	(0·25 — 0·87)	PNP
	0·53 ± 0·30 (3)	(0·39 — 0·78)	4-MU
α-L-fucosidase	0·75 ± 0·23 (5)	(0·42 — 1·17)	PNP
β-D-fucosidase	0·59 ± 0·19 (2)	(0·40 — 0·78)	PNP
β-D-N-acetylglucosaminidase	8·3 ± 1·8 (4)	(5·4 — 10·4)	PNP
Acid phosphatase	30·8 ± 8·9 (7)	15·5 — 46·4)	PNP
	16·7 ± 5·1 (4)	(9·0 — 21·8)	αNP

The enzyme activities were assayed as described in the text, using either p-nitrophenol compounds or 4-methylumbelliferone compounds as substrates. Activities were expressed with their standard deviations. ( ) denotes the number of skin samples analysed. PNP stands for the p-nitrophenol compounds, and 4-MU for 4-methylumbelliferone compounds; αNP stands for α-naphthyl phosphate.

2. *pH Optima of Various Acid Hydrolases*

The pH optima were measured in 0·2 mol/l acetate or in 0·2 mol/l citrate buffers with pH values ranging from 3·5 to 5·5. The results are shown in Table II and Fig. 1, 2, 3, 4. It has been reported that β-glucuronidase is inhibited by citrate ions (Mills *et al.*, 1953; Woollen and Walker, 1965), and β-N-acetyl-glucosaminidase by acetate ions (Pugh *et al.*, 1957). These enzymes show different pH optima when these buffers are compared (Robinson *et al.*, 1967). Epidermal β-N-acetyl-glucosaminidase shows similar pH optima (around 4·0) in both acetate and citrate buffers (Table II and Fig. 1).

TABLE II.—*pH Optima of Acid Hydrolases*

Enzyme	Optimal pH	Buffer	Substrates
α-D-glucosidase	3·7	0·2 mol/l acetate	4-MU derivative
β-D-glucosidase	4·8	0·2 mol/l citrate	4-MU derivative
β-D-glucuronidase	4·5	0·2 mol/l citrate	4-MU derivative
	4·6	0·2 mol/l acetate	4-MU derivative
α-D-mannosidase	4·3	0·2 mol/l citrate	4-MU derivative
α-D-galactosidase	3·8	0·2 mol/l acetate	4-MU derivative
β-D-galactosidase	4·5	0·2 mol/l citrate	4-MU derivative
β-D-N-acetylglucosaminidase	3·7	0·2 mol/l acetate	4-MU derivative
	3·9	0·2 mol/l citrate	4-MU derivative
Acid phosphatase	5·0	0·2 mol/l citrate	PNP
	6·0	0·2 mol/l citrate	αNP

The enzyme activities were assayed as described in the text. PNP and 4-MU denote the same as in Table I.

Acetate was slightly inhibitory at lower pH, while citrate was more inhibitory at pH higher than 4·5. There was no appreciable difference in pH optimum and the relative activities of epidermal β-glucuronidase when the 2 buffer systems were compared (Table II).

3. *The Effect of Various Substrates Concentrations on the Enzyme Activities*

Most of the acid hydrolases showed similar K_m values for their substrates, using either 4-methylumbelliferone or p-nitrophenol compounds as substrates in 0·2 mol/l acetate buffer. They are in the range of 0·3–0·5 mmol/l (Table III). β-N-acetyl-glucosaminidase and β-glucuronidase had higher K_m values (1·0 mmol/l) (Table III). A high K_m for β-N-acetylglucosaminidase compares with that of rat kidney enzyme (Robinson *et al.*, 1967), and may be due to poor substrate solubility.

TABLE III.—*K_m for Substrate*

Enzyme	K_m value	Remarks
β-D-glucosidase	$5{\cdot}0 \times 10^{-4}$ mol/l	4-MU
β-D-glucuronidase	$1{\cdot}0 \times 10^{-3}$ mol/l	4-MU
β-D-mannosidase	$5{\cdot}0 \times 10^{-4}$ mol/l	4-MU
α-D-galactosidase	$5{\cdot}0 \times 10^{-4}$ mol/l	4-MU
β-D-galactosidase	$5{\cdot}0 \times 10^{-4}$ mol/l	4-MU
β-D-N-acetylglucosaminidase	$1{\cdot}0 \times 10^{-3}$ mol/l	4-MU
Acid phosphatase	$3{\cdot}0 \times 10^{-4}$ mol/l	α-NP
	$2{\cdot}9 \times 10^{-4}$ mol/l	PNP

The enzyme activities were assayed as described in the text. 4-MU, PNP and αNP denote the same as in Table I and II.

4. *Cross-Reaction Between β-D-galactosidase and β-D-glucosidase*

Robinson *et al.* (1967), separated β-galactosidase activity of rat kidney homogenate into 2 bands by gel electrophoresis. Because their less active, faster-migrating band also contained β-glucosidase activity, they concluded that, although the major part of rat kidney β-galactosidase is highly specific, a less specific type is also present. This possibility was examined in epidermal homogenates using p-nitrophenyl-β-D-glucoside and p-nitrophenyl-β-D-galactopyranoside as substrates (Table IV). β-galactosidase and β-glucosidase activities with their own substrates were 1·0 and 0·24 nmol/min/mg protein respectively. When the 2 substrates were combined, the activity was 1·27 nmol/min/mg protein. This

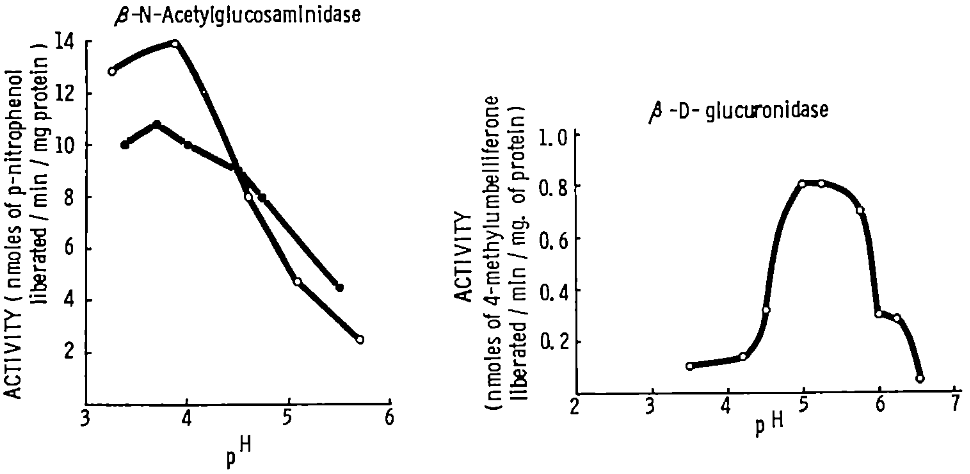

FIG. 1

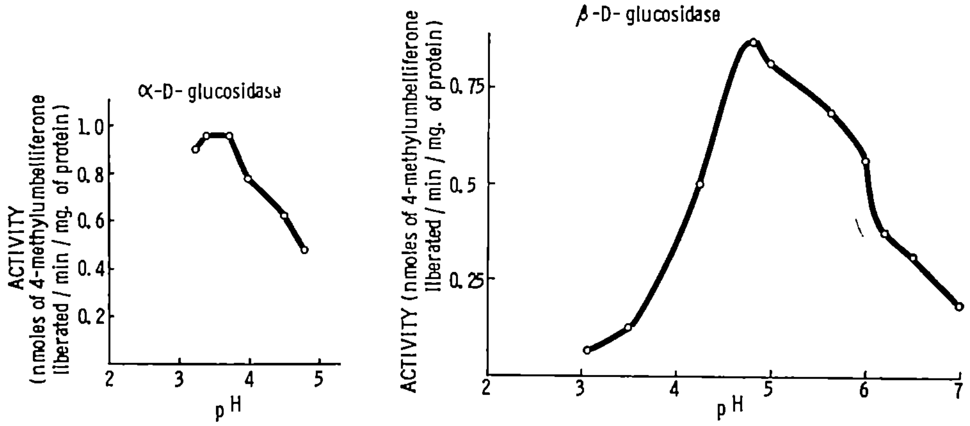

FIG. 2

FIG. 1, 2, 3, 4.—The pH curves for various acid hydrolases. The enzyme activities were assayed as described in the text. Open circle for β-N-acetylglucosaminidase in Fig. 1 is the curve for citrate buffer, whereas the closed circle is for acetate buffer. Acid phosphatase activity was measured using α-naphthyl phosphate (open circle) and p-nitrophenol phosphate (closed circle) as substrates.

TABLE IV.—*Cross-Reaction between β-D-galactosidase and β-D-glucosidase*

Epidermal homogenate	Activities (nm/min/mg protein)	Substrates
100 μl	1·00	p-Nitrophenyl(PNP)-β-D-galactopyranoside
100 μl	0·24	PNP-β-D-glucoside
100 μl	1·27	PNP-β-D-galactopyranoside + PNP-β-D-glucoside

β-Galactosidase and β-glucosidase activities were assayed individually, as described in the text. An assay tube containing both PNP-β-D-galactopyranoside and PNP-β-D-glucoside was also run.

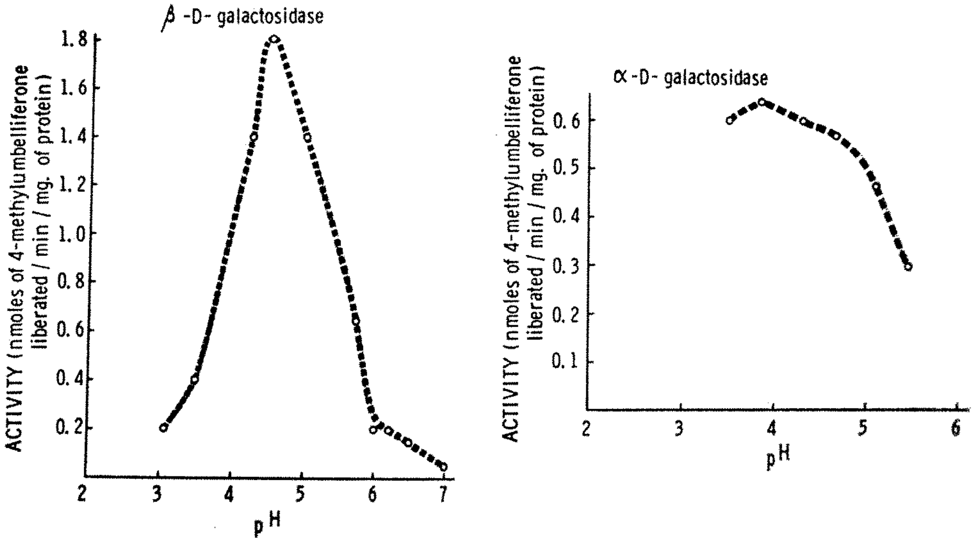

FIG. 3

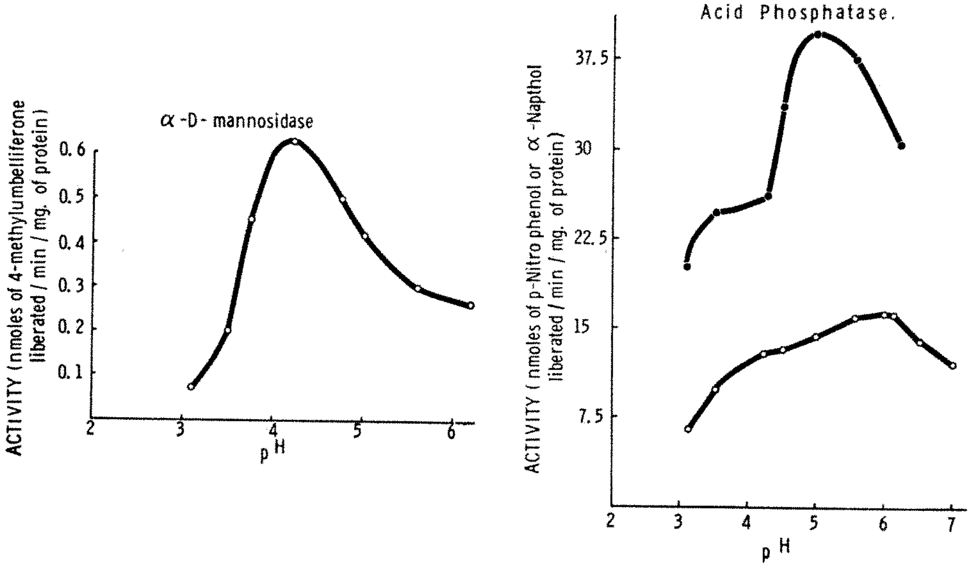

FIG. 4

suggests that epidermis contains 2 independent enzymes, with little cross reaction between these substrates.

5. *Lysosomal and a Non-lysosomal Acid Phosphatase*

A lysosomal and a non-lysosomal acid phosphatase have been suggested in normal human epidermis (Braun-Falco and Rupec, 1967; Mishima, 1964). Using 5% homogenates in 0·25 mol/l sucrose, fractional centrifugation was carried out, and the results are shown in Table V. Both PNP acid phosphatase and α-naphthyl acid phosphatase showed a bimodal-activity pattern, suggesting a lysosomal and a non-lysosomal localization. G-6-P DH activity, used as a marker, was localized to the cytoplasm. Unlike rat kidney β-glucuronidase, which is a lysosomal enzyme (Price and Dance, 1967), epidermal β-glucuronidase appears to be present, both in the lysosomes and free in the cytoplasm.

TABLE V.—*Acid Phosphatase Activity in Various Fractions*

	Whole homogenate	700 *g* precipitate	700 *g* supernatant	50,000 *g* precipitate	50,000 *g* supernatant
Protein	18·0	13·0	3·7	0·08	3·2
% Recovery		72	21	0·4	18
PNP acid phosphatase	47·2	29·1	103	198	97
% Recovery		44	45	1·9	36
αNP acid phosphatase	24	18	62	57	57
% Recovery		50	53	1·1	42
β-Glucuronidase	1·7	0·8	5·7	7·5	6·5
% Recovery		33	70	2	69
G-6-P dehydrogenase	6·6	2·5	27	0	28
% Recovery		28	84	0	76

The enzyme activities were assayed as described in the text and they were expressed as nmol/min/mg protein.

Previous papers (Neil and Horner, 1962; Abul-Fadl and King, 1949) indicate that alloxan is highly inhibitory to non-lysosomal enzyme, while tartrate is more inhibitory to lysosomal enzyme. This difference in inhibition was questioned by recent comprehensive work by Sloat *et al.* (1969). They found no difference in response to sodium tartrate, sodium fluoride, formaldehyde and alloxan when the supernatant and mitochondrial-lysosomal fractions were compared.

Inhibition of acid phosphatase by 0·1 mol/l alloxan and 0·2 mol/l sodium tartrate in the 50,000 *g* supernatant and precipitate (lysosomal fraction), using p-nitrophenol and α-naphthol compounds as substrates, is shown in Table VI.

TABLE VI.—*Inhibition of Acid Phosphatase by Alloxan and Tartrate*

	50,000 *g* Supernatant		50,000 *g* Precipitate	
	0·1 mol/l Alloxan	0·2 mol/l Tartrate	0·1 mol/l Alloxan	0·2 mol/l Tartrate
PNP acid phosphatase	25	88	37	85
αNP acid phosphatase	72	85	84	86

Numbers denote the % inhibition.

The enzyme preparations (50,000 *g* supernatant and 50,000 *g* precipitate) were incubated for 20 min at room temperature with 0·1 mol/l alloxan and 0·2 mol/l tartrate. After the incubation, the enzyme activities were assayed as described in the text. Appropriate controls were also run.

With p-nitrophenol phosphate as substrate, sodium tartrate was more inhibitory to the enzyme in both fractions; 84% to 88% of the activities were inhibited by 0·1 mol/l sodium tartrate, while only 25% (in supernatant) and 37% (in precipitate) were inhibited by 0·2 mol/l alloxan. With α-naphthyl acid phosphate as substrate, 85% of the activities were inhibited by tartrate and 84% (in precipitate) to 72% (in supernatant) were inhibited by alloxan. When PNP substrate was used, alloxan was less inhibitory than tartrate. There appeared to be no specific inhibitory effect of tartrate or alloxan on the 2 fractions studied.

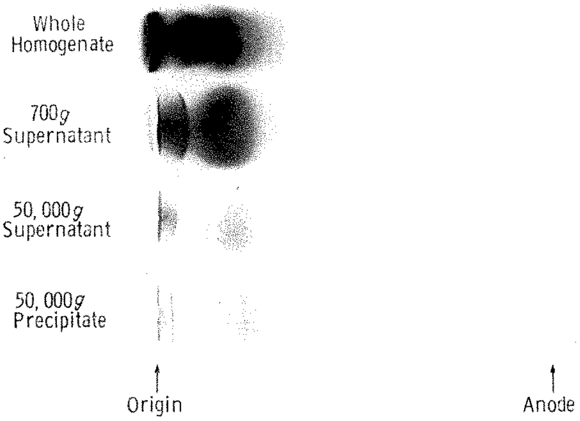

Fig. 5.—The electrophoretic pattern of acid phosphatase in the various cell fractions. The electrophoresis and staining were carried out as described in the text.

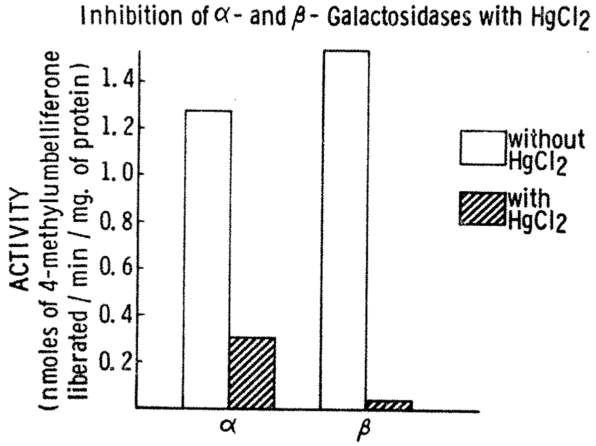

Fig. 6.—The inhibition of α- and β-galactosidases with 3 mmol/l $HgCl_2$. The enzyme activities were assayed as described in the text; α and β denote α-galactosidase and β-galactosidase respectively.

To determine whether the lysosomal and cytoplasmic enzymes from the epidermis are the same enzyme in different subcellular locations or different enzymes, an agarose gel electrophoresis was done. Two distinct anodally-migrating bands were demonstrated in the whole homogenates, 700 *g* supernatants, and 50,000 *g* supernatants and precipitates (Fig. 5). There was no qualitative difference in the fractions studied. It appears likely, therefore, that the acid phosphatase from the epidermis is the same enzyme in different intracellular location.

6. *Inhibition of D-galactosidases by* $HgCl_2$

Lee and Wacek (1970) reported a marked difference in the inhibitory effect of $HgCl_2$ on α- and β-D-galactosidases purified from *Aspergillus niger*. Their results showed that 3 mmol/l $HgCl_2$ was strongly inhibitory to α-D-galactosidase (95% inhibition), while only 3% of β-D-galactosidase activity was inhibited by the same concentration. Epidermal β-galactosidase was inhibited more strongly than α-galactosidase, and the difference was not as great as that found by Lee and Wacek (1970); 97·4% of β-galactosidase and 77% of α-D-galactosidase activities were inhibited by 3 mmol/l $HgCl_2$ under our experimental conditions (Fig. 6). Whether this difference is due to the different source of the enzyme or to the different degree of enzyme purification is unknown.

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COMPARATIVE STUDY OF ^{3}H-THYMIDINE LABELLING OF THE DERMAL INFILTRATE OF SKIN ALLERGIC AND IRRITANT PATCH TEST REACTIONS IN MAN

J. M. LACHAPELLE

Summary.—The ^{3}H-Thymidine (^{3}HT) nuclear labelling index of " round " cells of the dermal infiltrate in allergic and irritant patch test reactions in man has been compared. The skin of 15 positive allergic patch test reactions (to different allergens) and of 15 positive irritant reactions (5 to croton oil, 5 to potassium hydroxide and 5 to white spirit) was biopsied 48 hours after the application of the chemical. Thin slices of each specimen were incubated for 60 min in the presence of ^{3}HT, then fixed and prepared for light microscopy radioautography. Two thousand " round " cells of the infiltrate were counted in each specimen.

The mean values of the ^{3}HT labelling indices ($\pm$ S.E.) were $2{\cdot}91 \pm 0{\cdot}18\%$ in the allergic group and $0{\cdot}58 \pm 0{\cdot}06\%$ in the irritant group. The difference between the 2 means was highly significant ($P < 0{\cdot}01$). There was no significant difference between the labelling indices of the 3 irritants ($P > 0{\cdot}05$). All the labelled cells were mononucleated.

This method could be useful to differentiate clinically dubious allergic and irritant reactions.

ONE of the problems that frequently occur in occupational dermatology is related to the differentiation between irritation of the skin and allergic contact dermatitis. If obvious in some cases, it remains a puzzle in many others. The same diagnostic difficulties may be encountered when interpreting patch test reactions.

The importance of a clear-cut distinction between these reactions is fundamental in clinical work.

Many criteria, clinical, histological or cytological, have been proposed for establishing more precisely the aetiological (allergic or irritant) mechanism in doubtful cases, but none has proved itself entirely reliable.

The purpose of this study is to evaluate the potential usefulness of a new differential criterion, i.e. the *in vitro* ^{3}H-thymidine pulse labelling index of " round " cells of the dermal infiltrate in allergic and irritant patch test reactions, using the light microscopy radioautographic technique.

MATERIAL AND METHOD

Fifteen allergic and 15 irritant patch test reactions obtained from different patients have been compared (Table I). Each test was performed on the upper part of the back, using the Al-Test Imeco for occlusion. The patch was left under occlusion for 48 hours and read 1 hour after removal of the material.

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TABLE I.—*Patch Test Reactions*

15 Allergic			15 Irritant		
Substance	Concentration (%)	Vehicle	Substance	Concentration (%)	Vehicle
Potassium dichromate (2)	0·5	Petrolatum	Croton oil (5)	50	Olive oil
Nickel sulphate (3)	5	Petrolatum	Potassium hydroxide (5)	3	Water
Cobalt chloride (1)	1	Petrolatum	White spirit (5)		As is
Formaldehyde (2)	2	Water			
Procaine HCl (1)	2	Water			
Sulphanilamide (2)	5	Petrolatum			
Neomycin sulphate (2)	20	Petrolatum			
Promethazine (1)	2·5	Water			
Iodochlorhydroxyquinoline (1)	5	Petrolatum			

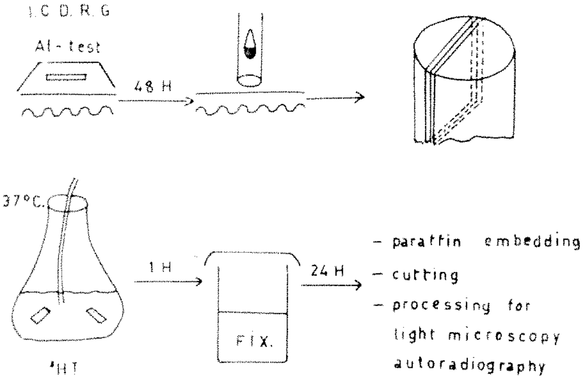

FIG. 1.—Experimental procedure.

Among the allergic reactions, only those which were recorded as ++ according to the scale recommended by the International Contact Dermatitis Research Group (Wilkinson *et al.*, 1970) have been considered for this study.

The methodology of the experiment has been summarized in Fig. 1. The site of each positive patch test reaction (irritant or allergic) was biopsied (4 mm punch biopsy). The biopsies were cut into very thin slices (< 1 mm), which were incubated at 37° C for 1 hour in a culture medium containing 2 μCi of ^{3}H-thymidine (^{3}HT, specific activity 10 Ci/mmole; concentration 1 mCi/ml; Centre d'Energie Nucléaire, Mol, Belgium) according to the technique of Lachapelle and Gillman (1969). They were then fixed in 4% buffered formalin for 24 hours, paraffin embedded, cut and processed for light microscopy radioautography. The sections were stained with H. and E. or nuclear fast red.

In each specimen, 2,000 nuclei of the " round " cells forming the dermal infiltrate were counted. The counts were limited to the cells of the well-defined perivascular infiltrates. They did not include cells which were moving from the perivascular spaces towards the epidermis or were exocytic, i.e. which were located between epidermal cells or in intraepidermal vesicles. The ^{3}HT nuclear labelling index, i.e. [(number of labelled cell nuclei/total number of counted cell nuclei) × 100], was determined in all specimens.

RESULTS

The most important finding is the relatively high number of ^{3}HT pulse labelled nuclei of " round " cells of the perivascular infiltrate in the allergic patch test

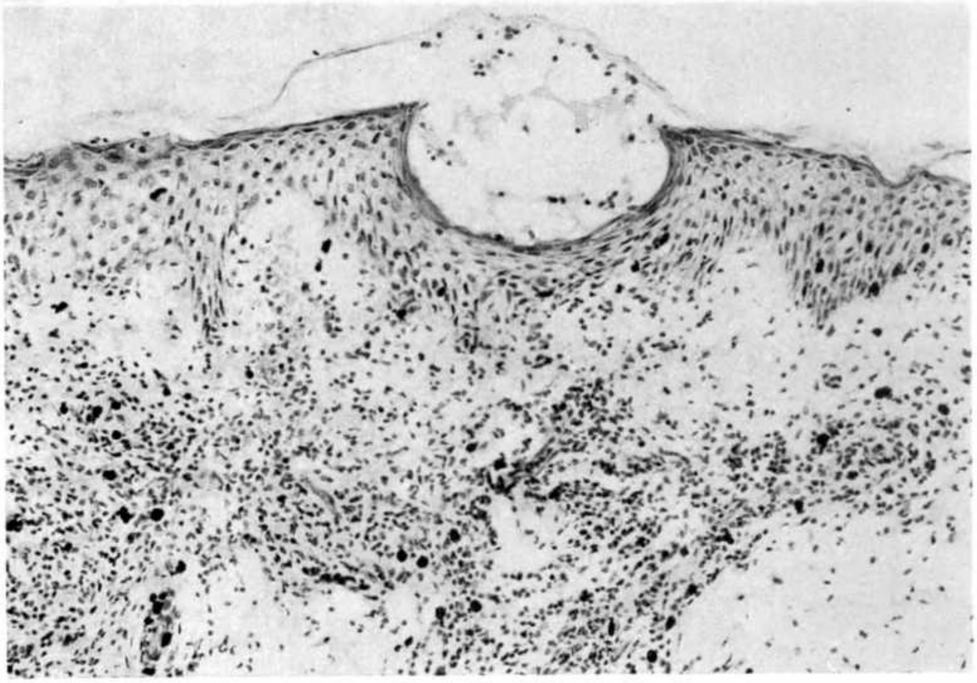

FIG. 2. *In vitro* ^{3}HT labelling of a typical allergic patch test reaction (to neomycin) at 48 hours. A relatively high number of cells of the dermal infiltrate are labelled (H. and E. × 105).

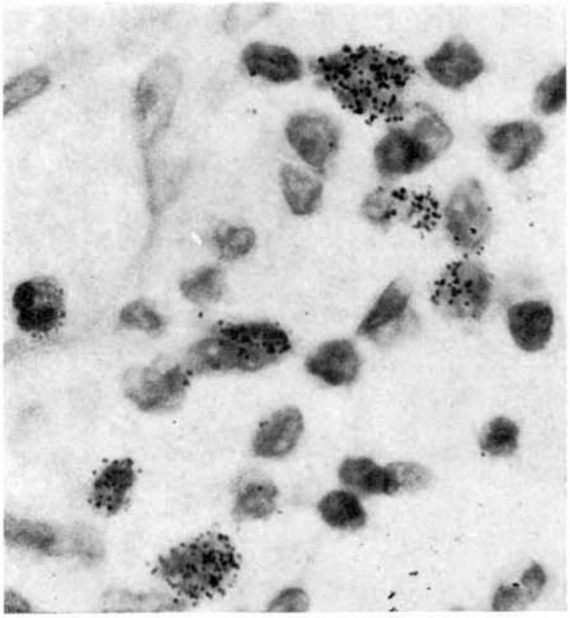

FIG. 3. Allergic patch test reaction (to sulphanilamide) at 48 hours. Many " round " cells of the infiltrate around a blood capillary are labelled (H. and E. × 725).

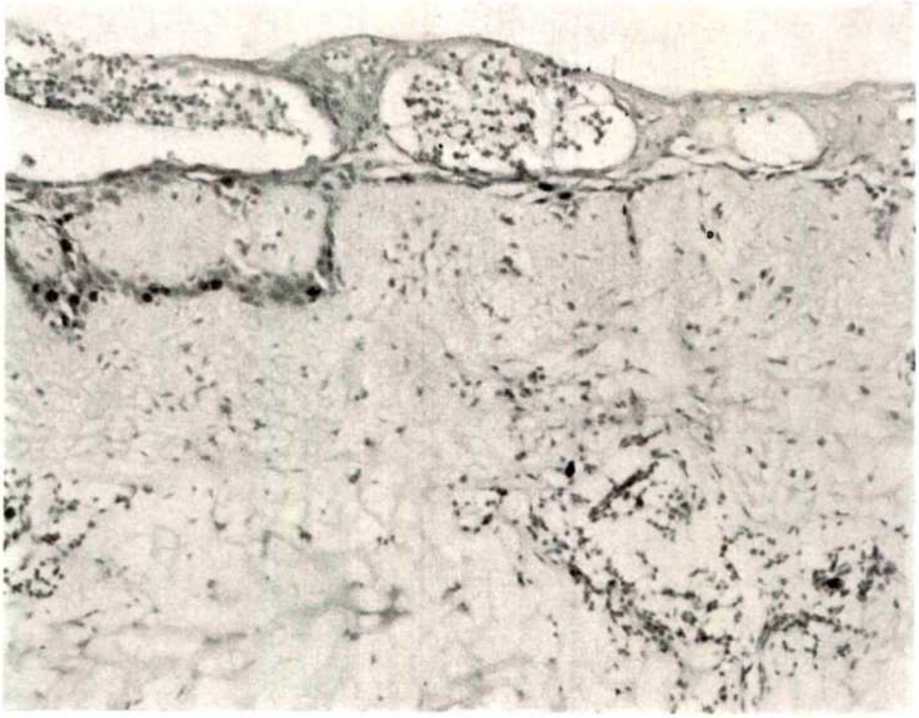

FIG. 4.—*In vitro* ^{3}HT labelling of a typical irritant patch test reaction (to white spirit) at 48 hours. Very few cells of the dermal infiltrate are labelled (nuclear fast red. × 125).

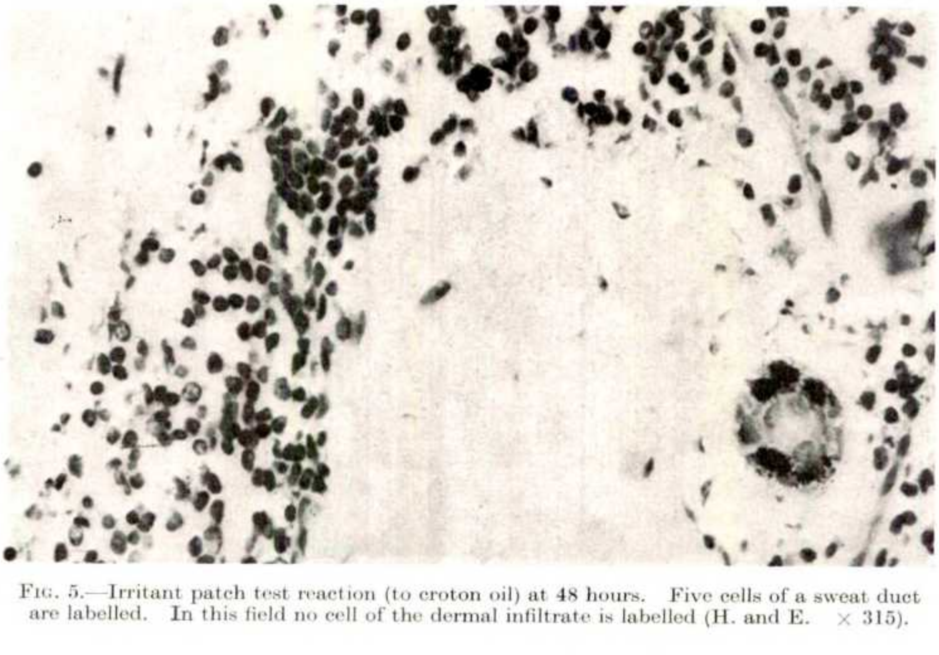

FIG. 5.—Irritant patch test reaction (to croton oil) at 48 hours. Five cells of a sweat duct are labelled. In this field no cell of the dermal infiltrate is labelled (H. and E. × 315).

reactions (Fig. 2, 3). By contrast, very few " round " inflammatory cells can be found labelled in the perivascular infiltrate of the irritant patch test reactions (Fig. 4, 5). The mean values of the ^{3}HT labelling indices (± S.E.) are 2·91 ± 0·18% in the allergic group and 0·58 ± 0·06% in the irritant group (Table II). The difference between the 2 means is highly significant ($P < 0{\cdot}01$).

TABLE II.—*Skin Patch Test Reactions. L.I. of " Round " Cells (*) of the Dermal Infiltrate*

	Type of reaction	
	Allergic	Irritant
Number of cases	15	15
L.I. ($\bar{x}$ ± S.E.)	2·91±0·18%	0 58±0·06%
	$P < 0$ 01 (t test)	

* Two thousand cells counted in each specimen.

TABLE III.—*Irritant Reactions. L.I. of " Round " Cells (*) of the Dermal Infiltrate*

Irritant	Number of cases	L.I. ($\bar{x}$ ± S E.)
Potassium hydroxide	5	0·74±0·11%
White spirit	5	0·48±0·08%
Croton oil	5	0·53±0·13%
	Analysis of variance $P > 0{\cdot}05$	

* Two thousand cells counted in each specimen.

The labelling indices of the 3 irritants used for this study, i.e. croton oil, white spirit and potassium hydroxide, are compared in Table III; they do not show any statistically significant difference ($P > 0{\cdot}05$).

All labelled cells are mononuclear cells, but on histological sections it is impossible to determine whether they are monocytic or lymphocytic in origin.

DISCUSSION

In previous studies (Lachapelle and Gillman, 1969; Lachapelle, 1970) we have proved the validity of the *in vitro* method for ^{3}HT labelling of human skin. It has been assumed that this method provides similar results to those obtained after a single intravenous injection of ^{3}HT. We can therefore assess that the labelling indices obtained after an *in vitro* pulse of ^{3}HT are identical to those which could be obtained by *in vivo* labelling techniques.

The mean value of the ^{3}HT L.I. of " round " cells of the dermal infiltrate (2·9%) that we have obtained in allergic patch test reactions by human subjects is somewhat inferior to the figures of different authors analysing reactions of delayed hypersensitivity in rats or guinea-pigs.

Indeed, Kosunen *et al.* (1963) found 5–10% labelled " lymphoid " cells in rats sensitized to paraphenylene diamine or bovine serum albumin; and, in guinea-pigs sensitized to tuberculin or diphtheria toxoid, at different times after challenge with the appropriate substance. Spector (1967), in a similar series of experiments, found a 4% L.I. in the cells of the dermal infiltrate.

Groth (1967), challenging at 12, 24 and 48 hours guinea-pigs sensitized to dinitrochlorobenzene, found a 5% flash L.I. in lymphoid cells infiltrating the epidermis. Lidén (1967), using the same experimental model, found 5–7% labelled " lymphoid " cells infiltrating the dermis.

In recent experiments of sensitization in guinea-pigs, we were able to confirm these figures from the literature (Lachapelle, unpublished data).

The lower L.I. obtained in man could be related to differences in the rate of reaction between different animal species, or possibly to the fact that we have used different, less potent allergens in our study than those usually used for animal studies (i.e. D.N.C.B., diphtheria toxoid . . .).

No effort has been made in the present work to classify the labelled " round " cells of the infiltrate into lymphocytes, monocytes or histiocytes. A distinction of such a type, based only on morphology, would be of limited interest. A much more important approach would be to try to obtain a distinction between labelled cells, according to their possible physiological functions (such as antibody production, phagocytosis, etc.). Such a trial (but not combined with ^{3}HT labelling) has been made by Turk (1967). Work is now in progress in our laboratory to elucidate this question.

The most important finding that arises from the present study is the dramatic difference between labelling indices in allergic and irritant patch test reactions. It has to be emphasized that this comparison is made from typical allergic and typical irritant reactions. The distinctive criterion between both reactions could prove to be a useful additional tool to distinguish between doubtful allergic and irritant reactions. Its reliability, as compared to other well known distinctive criteria, in such doubtful cases, must be confirmed.

I am very grateful to Miss F. D. M. de Cock for preparing the radioautographs and to Mrs C. E. L. Schoonjans for secretarial help.

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DIRECT IMMUNOFLUORESCENT STUDY OF THE SKIN ON OCCURRENCE OF COMPLEMENT IN PEMPHIGUS

TH. VAN JOOST, R. H. CORMANE AND K W. PONDMAN

Summary.—In 6 cases of pemphigus (4 pemphigus vulgaris, 1 pemphigus vegetans and 1 pemphigus erythematosus) the direct immunofluorescent method (DIF) revealed various complement factors, as there are $C1_q$, C4 (β_1E), β_1A and α_2D, localized in the intercellular spaces of the epidermis of the skin or mucosa immediately next to the bulla.

Immunoglobulin and complement were shown together in the prickle cell layer of the epidermis, while complement staining alone usually could be shown in the intercellular spaces of basal cell layers and in the junctional zone.

The possibility that antibody-mediated complement deposition and/or alternate pathway activation may play a role in the underlying mechanism in pemphigus is discussed.

DIF STUDIES of biopsy specimens of lesional skin in pemphigus revealed *in vivo* binding of pemphigus antibody (IgG) distributed in a pattern confined to the intercellular spaces of the epidermis (Beutner and Jordon, 1964; Chorzelski *et al.*, 1966). Cormane and Chorzelski (1967) first described, with the DIF method, *in vivo* participation of complement at the same tissue sites using the so-called anticomplement serum.

Cormane *et al.* (1970) pointed out that, if pemphigus lesions or perilesional skin of the blisters were examined with epi-illumination, using the above mentioned anticomplement serum, complement also appeared to be present in the vessel walls and in the dermis. In contradiction to these findings, other authors (Jordon *et al.*, 1971, a and b) reported recently that they were not able to demonstrate *in vivo* presence of complement in the intercellular spaces of lesional skin in pemphigus using an antiserum against the third component of complement.

The question as to whether or not the pemphigus autoantibodies play a pathogenetic role in the disease has been discussed in various reports (Beutner *et al.*, 1965; Chorzelski *et al.*, 1966; Peck *et al.*, 1968). The close relationship between the disease activity in pemphigus and the titre of circulating antibodies directed against the intercellular spaces is in favour of such a role. Sams and Jordon (1971), however, reported a study in which transfusion of high-titre human pemphigus plasma into 4 monkeys failed to produce lesions. In this experiment the authors were not able to demonstrate presence of C3-globulin or fragments in the intercellular spaces of these monkeys with the DIF method, although the same method revealed binding of immunoglobulins (IgG) at the same sites.

In pemphigus vulgaris it is of interest that electron microscopic studies revealed not only alterations in the region of the intercellular spaces, but also changes of the basement membrane (Braun-Falco and Vogell, 1965, a and b).

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Schreiner and Wolff (1969) showed in an electron microscopic cytochemical study the existence of a certain permeability for low molecular weight proteins in the basal membrane zone of normal human skin. Inderbitzen and Grob (1967) reported that, in rabbits hyper-immunized with epithelial antigen, acantholysis occurred when " a barrier between epidermis and dermis (plasma) " was broken down after artificial traumatizing of the skin by application of sodium lauryl sulphonate and subsequent exposure to dry ice. Kint and Geerts (1971) found in the cases examined with pemphigus vulgaris that the basement membrane showed inconstant features. It is often broadened or unsharp, or even lacking. The fine fibrils connecting the basement membrane to the cell membrane are not altered. Results in this study may raise the question as to whether complement factors or products are involved in the process, inducing an abnormal exchange between dermis and epidermis and the blister formation in pemphigus.

MATERIALS

Four patients with pemphigus vulgaris, one patient with pemphigus vegetans and one patient with pemphigus erythematosus (Senear–Usher syndrome) were subjected to direct immunofluorescent skin study (DIF). The diagnoses were based on the clinical and laboratory findings. Biopsies of skin of 10 adult controls were subjected to the same procedure.

By means of the indirect immunofluorescence technique (IIF), circulating pemphigus autoantibodies of the IgG type were demonstrable in the sera of the patients, varying in titre from 1/16 to 1/320 (serum of Patient V (L), however, was not available). The investigations were performed in untreated cases and in cases in which, in spite of a maintenance therapy with corticosteroids, typical pemphigus lesions occurred. DIF method was also carried out in the clinically normal skin at a great distance from the lesions (extensor surface of the forearm) in a case in which only the mucous membrane of the mouth was involved. In the case of pemphigus erythematosus, both clinical and histological features of pemphigus and lupus erythematosus were present, and both manifestations were apparently triggered by exposure to sunlight.

TABLE I.—*Patients Studied by DIF*

Patient No.	Diagnosis	Affected area	Age	Sex	Biopsy
I (V)	P. vulgaris	Buccal mucosa	63	F	Before treatment
II (G-S)	P. vulgaris	Skin	78	F	Before treatment
III (W–S)	P. vulgaris	Buccal mucosa	76	F	During treatment with corticosteroids
IV (V–K)	P. vulgaris	Buccal mucosa and skin*	60	F	During treatment with corticosteroids
V (L)	P. vegetans	Skin	68	M	Before treatment
VI (W–B)	P. erythem.	Skin	72	F	Before treatment

* Biopsy was taken from the skin.

The Preparation of the Skin Sections

Biopsies were collected from the pemphigus patients visiting our clinic during the last 2 years. Punch biopsies (3–4 mm) were taken from the skin or mucosa immediately next to the bullae (perilesional skin), in one case at a great distance from lesions. The biopsies were stored in an ice box (−90°C).

Reagents Used for the in vivo *IF Studies (DIF)*

For the *in vivo* localization of complement factors the double-layer technique had to be used because no FTC-labelled reagents were available.

Anti-complement serum was obtained by immunization of rabbits with antigen-antibody complexes at equivalence (egg-albumin/rabbit-anti-egg albumin), which were previously incubated with fresh human AB serum (methods of preparation are detailed in literature (Pondman *et al.*, 1960; Rosenfield, 1960). This contained precipitating antibodies against Cl_q, C4 (β_1E), β_1A, α_2D and C5 (β_1F).

The presence or absence of antibodies against Cl_r, Cl_s and C6 through C9 could not be established because these antigens were not available.

Antisera Used for Detection of β_1E, β_1A, β_1C and α_2D

Detailed information with respect to the specificity and preparation of immune sera against complement components or fragments has recently been published by Engelfriet *et al.* (1970).

Antisera Used for Detection of Cl_q and β_1F

Anti-Cl_q serum was obtained by immunization of rabbits with antigen-antibody complexes (egg-albumin/rabbit-anti-egg-albumin, optimal complexes), previously incubated with 0·01 mol/l Na_3HEDTA containing fresh human serum. In precipitation tests it was shown to be specific for Cl_q; however, it is not certain that it does not contain agglutinating antibodies against complement substance other than Cl_q.

Anti-β_1F serum has the same disadvantage that it was shown to be specific only in immunodiffusion. It was prepared by immunization of rabbits with a "pure" β_1F globulin. The antiserum was made specific by absorption with heated fresh human serum. For the detection of the different complement factors in the second step (double layer method) an FTC-conjugated specific goat-anti-rabbit-antiserum was used. For the detection of IgG an FTC-conjugated specific rabbit-anti-human antiserum was used.

Swine antiserum against the Fc-fragment (Nordic) contains antibodies not only against the Fc-fragment, but also against the sub-fragment F′c. In testing a native IgG, this protein is precipitated by reaction with the heavy chains of IgG. In testing against a fragmented IgG containing the subfragment F′c, e.g. papain digest prepared in the presence of cysteine, it precipitates, apart from the fragment Fc, also the sub-fragment F′c, with an electrophoretic mobility from the α-2 region to that of albumin. An FTC-conjugated rabbit-antiswine antiserum was used in the second step (double layer method) for the detection of the Fc-fragment.

A normal rabbit serum was used as a control in the double layer technique.

*Characteristics of FTC-protein Conjugates**

Rabbit anti-human IgG/FTC (RAHuIgG/FTC batch 0–170): protein conc. 1%, $F/P = 2{\cdot}5 \times 10^{-3}$/mg (dil. 1/32).

Goat anti-rabbit/FTC (GAR/FTC batch 3–171): protein conc. 1·37%, $F/P = 2{\cdot}6 \times 10^{-3}$/mg (dil. 1/64).

Rabbit anti-swine/FTC (RASw/FTC batch 2–969): protein conc. 1·76%, $F/P = 8{\cdot}1 \times 10^{-3}$/mg (dil. 1/32).

The optimal dilution for various anti-complement sera and the anti-Fc serum appeared to be 1/16, for the rabbit anti-human IgG/FTC antiserum and the rabbit anti-swine/FTC antiserum 1/32 and for the goat anti-rabbit/FTC antiserum 1/64.

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* Nordic Pharmaceuticals and Diagnostics, Tilburg, Holland. The anti-complement serum and the anti-component and anti-fragment sera were prepared in the Central Laboratories of the Netherlands Red Cross Blood Transfusion Service, 125 Plesmanlaan, Amsterdam.

TABLE II.—*Occurrence of IgG, Fc-fragment and Complement Factors in Pemphigus (Skin Adjacent to the Lesions)*

Patient No.	IgG		compl		Cl_q		$\beta_1E(C4)$		β_1C		α_2D		β_1A		$\beta_1F(C5)$		Fc	
	ic	BMz	ic	BMz	ic	BMz	ic	BMz	ic	BMz	ic	BMz	ic	BMz	ic	BMz	ic	BMz
I (V)	+ +	—	+ + +	+ + +	+ +	—	+ +	+	(+)	—	+ + +	+ + +	+	—	—	—	+	—
II (G–S)	+ + +	—	+ + +	+	+	—	+	(+)	—	—	+ + +	+	+ +	—	—	—	+	—
III (W–S)	+	—	+	+	+	—	—	—	+	—	+	+ +	(+)	—	—	—	(+)	—
IV (V–K)	+	—	+ +	+	(+)	—	(+)	—	—	—	+ +	+	+	—	—	—	+	—
V (L)	+	—	+ +	+ + +	+	(+)	+	+ +	—	—	+	+ + +	+ +	—	—	—	—	—
VI (W–B)	+	—	+	+	—	—	—	—	—	—	+	+	+	—	—	—	+	—
VI (W–B)	+ +		+ +		(+)		+ +		(+)		+ + +		+ +		+		—	
Granular deposits in BM zone and dermis																		
I (V) unaff. skin	+	—	+	(+)	(+)	—	—	—	—	—	—	(+)	—	—	—	—	+	—

+ + + = strong positive. (+) = doubtful to weak positive.

Microscopy

The optical system for blue narrow-band excitation (with wavelengths between 470–490 nm) used in this study has been extensively described in a previous paper (Cormane and Szabo, 1970).

RESULTS

The results of this study indicate the *in vivo* presence of complement components and factors in the intercellular spaces of lesional skin of patients with pemphigus.

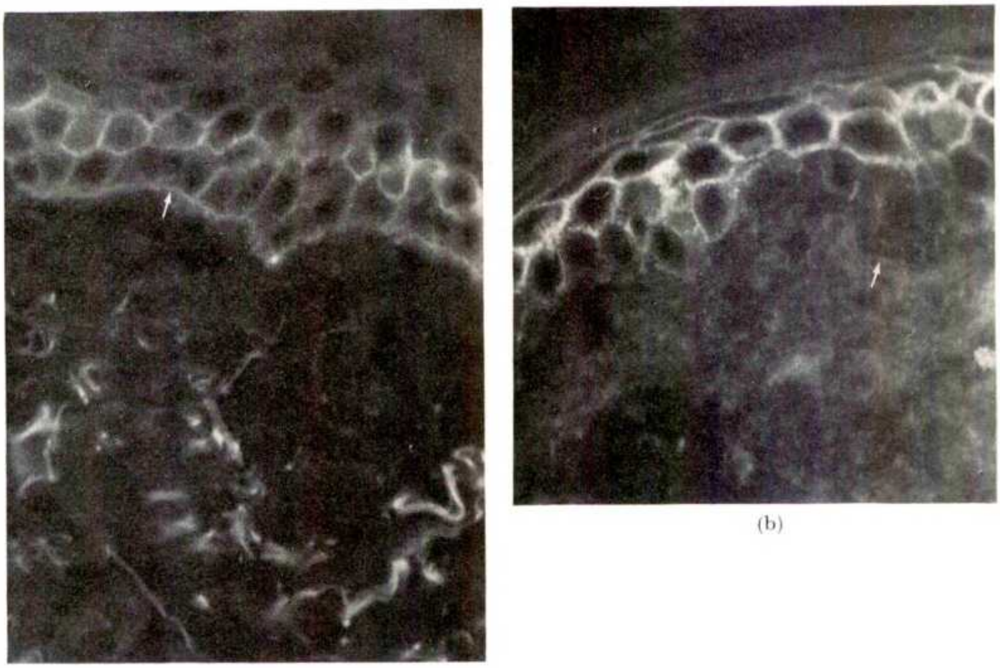
(a) (b)

FIG. 1.—(a) Blue narrow-band excitation, water immersion, DIF method (× 10). Pemphigus vulgaris (Patient II). Skin adjacent to the bullous lesion. Complement factor α_2D (× 50). Note the intercellular staining and the staining of the junctional zone. (b) Pemphigus vulgaris (Patient II). Skin adjacent to the bullous lesion. IgG/FTC (× 50). Note the bright intercellular staining pattern of the prickle-cell layer, with specific anti-IgG serum, and the partly different localization of staining with antisera against factor α_2D in the same biopsy. (The arrow in fig. 1b points to the negative BM zone.)

In one patient examined, IgG and complement (factor Cl_q) staining were also demonstrable in small amounts in a skin biopsy of the forearm, although only the buccal mucosae showed typical pemphigus lesions. In all 6 patients examined, *in vivo*-bound immunoglobulin (IgG) was present in the intercellular spaces of the epidermis. The presence of Fc-fragment in the same regions was shown in 5 of 6 cases. Complement factors α_2D, β_1A and Cl_q were most constantly present in the intercellular spaces.

The specific staining with anti IgG and anti Fc-antiserum was shown mainly in the "prickle cell layer" of the epidermis. With the specific anti-complement sera, however, the intercellular staining pattern was present mostly in the lower region of the epidermis, and in all 6 patients anticomplement staining of the junctional zone was demonstrable. In 2 patients (I and V) this staining pattern was similar, but not identical with that seen in pemphigoid (Pg) after DIF staining of lesional skin with an anti-human complement serum.

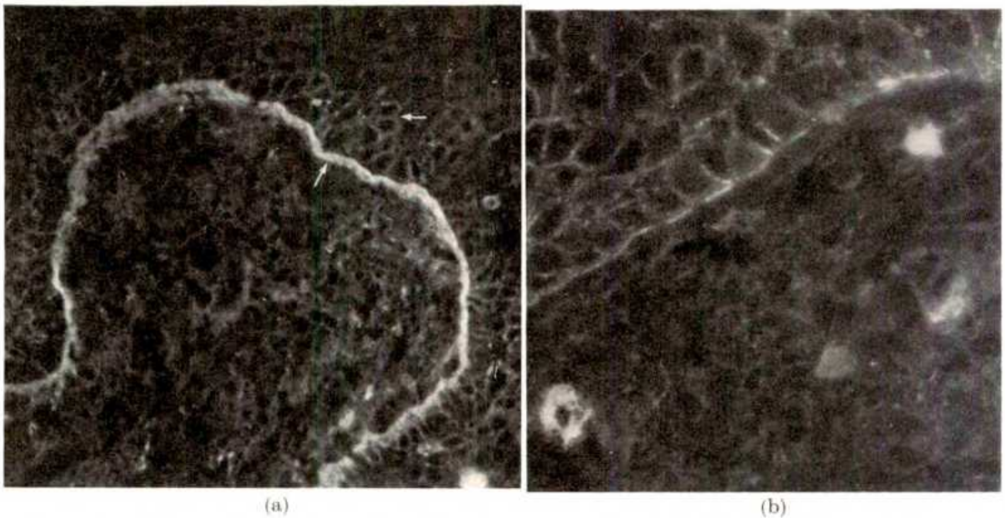
(a) (b)

FIG. 2.—(a) Pemphigus vegetans (Patient V). Skin adjacent to the bullous lesion. Complement (× 50). Note the mainly linear fluorescence of the junctional zone and the staining pattern of the intercellular spaces with anti-complement serum. (b) Pemphigus vegetans (Patient V). Skin adjacent to the bullous lesion. Complement factor β_1A (× 50). Note the intercellular fluorescence of the epidermal layers. The basal staining does not seem to be due to fluorescence of the basement membrane.

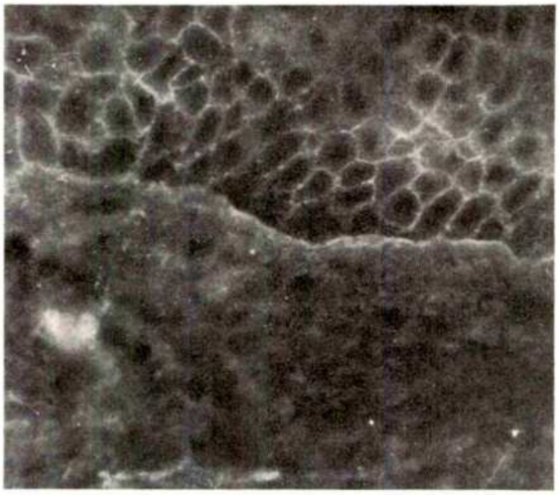

FIG. 3.—Pemphigus vulgaris (Patient IV). Skin adjacent to the bullous lesion. Complement factor α_2D (× 50). Note bright fine intercellular staining pattern. At the junctional zone a partly linear fluorescence is demonstrable.

In the other cases the complement staining pattern of the junctional zone was partly linear and partly of a more granular type. In the case of pemphigus erythematosus (VI), besides an intercellular staining pattern, a linear fluorescence of the basement membrane zone was not concomitant in some places with granular immunoglobulin deposits. It is admitted that the complement staining pattern of the junctional zone in this case may be considered as " a manifestation of both " pemphigus and LE. In our study it appears that α_2D fluorescence was strikingly concentrated in the basement membrane zone. In 2 cases the fourth component of complement (β_1E) was also present in this region in a linear pattern.

The vessel walls in the upper part of the papillary region showed in all 6 patients a more or less homogeneous staining pattern with the anticomplement-component sera. Fluorescence of the vessel walls, however, is not considered to be specific for one skin disease (Baart de la Faille-Kuyper and Cormane, 1968).

In none of our cases of pemphigus was specific fluorescence demonstrable in the intercellular spaces or the junctional zone with the antiserum directed against β_1F (C5). In the case of pemphigus erythematosus, however, granular deposits were detectable at the dermo-epidermal junction (dermal site) with all the anti-complement sera used.

Negative results were obtained after incubation with the normal rabbit serum and the goat-anti-rabbit/FTC antiserum, and also after incubation with the goat-anti-rabbit/FTC antiserum alone. No specific staining pattern could be shown with the specific anti-complement sera in the control skin biopsies examined.

DISCUSSION

Evidence is accumulating that tissue-injuring mechanisms in many diseases are mediated by complement or fragments of complement. Examples are the links of the complement system with leucotaxis (Ward *et al.*, 1965), blood clotting (Donaldson, 1968) and the capacity to increase the level of permeability of the vessel walls (Dias da Silva *et al.*, 1958). The complement system has also been shown to be involved particularly in diseases presenting some evidence of auto-sensitization (Lachmann *et al.*, 1962; Morse *et al.*, 1962). In pemphigus vulgaris, lesions have been observed to occur mainly in the absence of inflammatory cells (Lever, 1965). It is of note in this respect that antibodies which fix complement poorly or inefficiently do not induce neutrophil accumulation (Bloch *et al.*, 1963). The absence of the fifth component of complement (β_1F) in the pemphigus vulgaris patients examined in our study may be another relevant factor.

In pemphigus vulgaris Katz *et al.* (1970) found no relationship between the serum concentration of C3 and the severity of the disease. The authors concluded, however, that these findings do not exclude the pathogenic involvement of complement in pemphigus, since other complement components may play a role in the process of acantholysis.

From the observation that pemphigus antibodies have a relatively low reactivity with intercellular substances of normal human epidermis, it may be concluded that alterations of antigenic determinants present in the intercellular spaces of the epidermis of patients with pemphigus vulgaris occur prior to the antibody fixation (Cormane and Petzoldt, 1970; Cormane *et al.*, 1972). Results of this study show the presence of various fragments of complement components at the site of the intercellular spaces of the epidermis in the skin immediately surrounding the lesions in pemphigus. At this site all the tested complement components and fragments, except β_1E, were present, but apparently in different amounts. The fragment α_2D seemed to be most frequent. In one case (III) antigenically-intact C3 was demonstrated intracellularly. The possibility exists that different results in the literature in the demonstration of complement in the intercellular spaces of skin of pemphigus patients may be due partly to uncertainty about the exact specificity of the anti-C3 sera used in these studies. Furthermore, the impression was gained that, in the untreated cases (I, II, V), the complement staining pattern was more striking than in the cases in which, in spite of corticosteroid therapy, lesions occurred. Simultaneous presence of subfactor of Cl, the Cl_q component and the Fc fragment of IgG in the intercellular spaces of the examined skin is in favour of the " classic " complement-activating sequence in which C deposition is antibody-mediated. Complement fragments were also detectable in the basement membrane zone mostly in linear pattern of fluorescence. No distinct amounts of immunoglobulins were demonstrable in basement membrane zone, except in the case of pemphigus erythematosus. At the dermal site of the dermo-epidermal junction, however, immunoglobulin (IgG) and complement seemed to be diffusely fixed to fibres. In this respect it is reasonable to speculate whether the presence of complement fragments and the absence of distinct amounts of antigen-antibody complexes in the basement membrane zone justify the hypothesis of a " by-pass " of complement activation (alternate complement activation). Of interest in this respect is the conclusion drawn from immunofluorescence data supporting the theory of participation of the complement components in the inflammatory response of the blood vessel walls (Cormane *et al.*, 1971). The authors suggested the possible existence in the skin of porphyria cutanea tarda of a photodynamically-induced alternate pathway which may activate the complement sequence, independent of immunological reactions. The role of properdin and the effect of the C3-proactivator system in the underlying mechanism of tissue injury, especially in bullous dermatoses, merit further study. The results of electron microscopic studies in the literature, in which disturbance of the basement membrane zone can be shown in pemphigus, raise the question as to whether complement factors are involved in the process inducing an abnormal exchange between the dermis and epidermis.

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MEASUREMENT OF LIPID SYNTHESIS IN MOUSE AURICULAR SKIN CULTURED *IN VITRO*

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Summary.—Split-thickness mouse auricular skin has been cultured *in vitro* for up to 48 hours in the presence of ^{14}C-sodium acetate, ^{14}C-leucine, ^{14}C-isoleucine and ^{14}C-valine, and the incorporation of radioactivity into various tissue fractions measured in the heat-separated epidermis and dermis. Lipid fractions were analysed by chromatography. It was found consistently that the epidermis contained higher proportions of labelled sterol esters and lower proportions of labelled triglyceride than the corresponding dermis. The radioactive acyl moieties of certain lipid classes were further studied by gas-liquid radiochromatography. With ^{14}C-acetate as the tracer, sterol esters of both epidermis and dermis were seen to be enriched with respect to branched chain and odd-numbered chain fatty acids compared with triglycerides and phospholipids. With ^{14}C-leucine, which is the specific precursor of odd-numbered chain iso-branched acids, sterol esters contained the majority of this type of fatty acid, although the major part of the radioactive substrate was metabolized to ^{14}C-acetate and incorporated into normal, even-number chain saturated and unsaturated acids. With ^{14}C-valine, a specific precursor of even-number chain iso-branched acids, epidermal and dermal sterol esters again contained the majority of these acids, although there was evidence of some degradation of the valine to acetate, and incorporation into normal fatty acids. With ^{14}C-isoleucine, a specific precursor of odd-numbered chain anteiso-branched acids, virtually all of the precursor was degraded to ^{14}C-acetate, and the resultant radioactivity patterns were quite similar to those obtained with ^{14}C-acetate cultures *per se*. Slight traces of radioactive anteiso acids were detected in certain lipid classes, however.

SINCE the first observations by Srere *et al.* (1950) that skin was capable of synthesizing lipids in it own right, independent of the influences of other organs, there have been many reports on the types of lipids synthesized by skin, generally utilizing excised skin, cultured *in vitro*. Nicolaides and co-workers first demonstrated that human skin, incubated *in vitro* with ^{14}C-sodium acetate, produced labelled squalene, cholesterol and fatty acids (Nicolaides and Rothman, 1955; Nicolaides *et al.*, 1955). Griesemer and Thomas (1963) showed that lipid synthesis was different in various regions of the skin, and demonstrated later (Griesemer and Thomas, 1966) that the major fatty acids into which labelled acetate was incorporated by human skin and adipose tissue were palmitic, myristic and oleic acids, with smaller amounts of palmitoleic, stearic, linoleic and linolenic acids. Hsia and his group, more recently, have undertaken extensive studies on lipogenesis in cultured human skin (Vroman *et al.*, 1969; Hsia *et al.*, 1970).

Similar studies have been performed on skin from other species. Brooks *et al.* (1966) studied acetate incorporation into mouse skin, and Ziboh and Hsia (1969) have described rat skin lipogenesis. Gaylor (1963) showed that the metabolism

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of ^{14}C-acetate to squalene and sterols was different in rat epidermis compared with the sebaceous glands. Similarly, the rigorous chromatographic studies of Horlick and Avigan (1963) and Clayton *et al.* (1963) have outlined the pathways of sterol metabolism in rat skin.

Wheatley and his group (Lipkin *et al.*, 1965; Wheatley *et al.*, 1967) adopted a different approach to studying lipogenesis in skin by perfusing isolated flaps of dog thigh skin with radioactive acetate, and other specific precursors of certain fatty acids (e.g. isoleucine, for odd-numbered chain anteiso-branched acids; valine, for even-numbered chain iso-branched acids). Recently, Grigor *et al.* (1970) have monitored the synthesis of these types of fatty acids in rat skin lipids by injection of similar radioactive precursors.

The work of Wheatley is of particular significance since specific branched-chain fatty acids have been widely reported in skin. James and Wheatley (1956) first demonstrated the presence of different branched fatty acids in sebum, using gas-liquid chromatography. The work was corroborated by Haahti (1961). Nikkari and Haahti (1964) and Nikkari (1965) reported similar branched fatty acids in the rat, and Carruthers (1962) and Carruthers and Heining (1963) described these fatty acids in mouse skin lipids. It is now firmly established that many complex cutaneous lipids contain a whole spectrum of branched even- and odd-numbered fatty acids in addition to the variety of even-numbered normal saturated and unsaturated acids, which are characteristic of mammalian tissues in general.

We are interested in the nature and biochemistry of the lipids of skin, since these play a vital role in the barrier properties of the organ, which protects the animal from its environment. A method for culturing mouse ear skin *in vitro* has been developed in which the tissue survives for at least several days, with apparently full maintenance of normal histological appearance and biochemical function. Specific aspects of lipid synthesis have been studied in this system. Mouse ear skin has been cultured in the presence of tracer amounts of ^{14}C-sodium acetate, ^{14}C-leucine, ^{14}C-isoleucine and ^{14}C-valine, when several types of branched chain fatty acids were labelled, the most significant type being the even-numbered chain iso-branched series.

MATERIALS AND METHODS

Materials

Fatty acids.—Reference odd- and even-numbered normal fatty acids and iso- and anteiso-branched fatty acids were obtained from Applied Science Laboratories, State College, Pennsylvania, USA.

Radioactive precursors.—2-^{14}C sodium acetate and $^{14}C(U)$-leucine, $^{14}C(U)$-isoleucine and $^{14}C(U)$-valine were obtained from the Radiochemical Centre, Amersham, Bucks. Each labelled compound was dissolved in sterile 5% sodium bicarbonate solution, such that 0·1 ml contained 10 μCi radioactivity. For the sodium acetate solutions the specific radioactivity was 50 μCi/μmol and, for each of the 3 amino acids, about 300 μCi/μmol. In no instance was carrier added. These sterile solutions were added directly to the culture media as described below.

Mice.—In all experiments the ears from male black mice aged 4 weeks (strain C53BL) were used. The animals, which were not litter mates, were fed *ad libitum* and killed by CO_2 asphyxiation prior to removal of the ears.

Chemicals and solvents.—All chemicals and solvents were of the highest grade of purity available. Hexane, used in chromatography, was redistilled over $KMnO_4$. The sources of all other specialized reagents used are quoted at the appropriate point in the text.

Methods

Tissue culture.—Mouse-ear skin was cultured under conditions resembling those reported by Gillette and Findley (1958). After asphyxiation of the mice, their ears were swabbed with 70% ethanol and then excised. The ears were scraped with a scalpel blade to remove hair: any cartilage at the base of the ears was also removed. Shallow incisions were made in the dorsal surface of each ear with a blunt scalpel blade so as to allow a rectangle of whole split-thickness skin to be peeled off. The excised rectangles of skin, which generally measured approximately 6 mm × 4 mm were treated with antifungal and antibiotic agents by placing them, epidermal-side downwards, on a gauze soaked in a mixture of mycostatin (100 mg/l), dihydrostreptomycin sulphate (100 mg/l) and benzyl penicillin (2×10^5 units/l) in Hanks' phosphate-buffered saline (Hanks and Wallace, 1949) for 30 min. Mycostatin was obtained from E. R. Squibb and Sons, Liverpool; dihydrostreptomycin sulphate BP and benzyl penicillin BP were obtained from Dista Products Ltd., Liverpool. The skin from each ear was divided into 4 pieces, each measuring about 2 or 3 mm square, and placed dermal-side downwards in 10 ml Erlenmeyer flasks. The skin fragments (12–16 pieces, representing 4 mouse ears) were then floated by careful addition of 2 ml Waymouth's 752/1 medium (Waymouth, 1959) prepared from a 10 × concentrate (Burroughs, Wellcome and Co., London) and containing 10% (v/v) calf serum (Flow Laboratories Ltd., Irvine, Scotland). Requisite amounts of the radioactive precursor solutions under examination were added under aseptic conditions. The flasks were gassed for 1 min with 5% CO_2 in air and stoppered tightly with sterile bungs. The skin cultures were then incubated at 30°C for various times.

Separation of epidermis from dermis after culture of the tissue.—After the desired time of culture, the pieces of skin were removed from the radioactive media and rinsed extensively with ice-cold saline (0·9% w/v). The tissue rinsings were added to the original culture media and assayed for radioactivity subsequently. After rinsing, the skin pieces were stored in an ice-cold Petri dish prior to heat separation (Baumburger *et al.*, 1942). The epidermis was separated from the dermis by placing the skin, dermis downwards, on a hot plate at 55°C for 90 seconds, the tissues being kept moist during heating by covering with a droplet of saline solution. The epidermis was then removed from the dermis by gently scraping with a scalpel under a dissecting microscope. The separated epidermis and dermis samples were immediately extracted for radioactivity as described below.

Oxidation of radioactive precursors to $^{14}CO_2$.—To determine the proportion of the requisite precursors which was oxidized to CO_2 under similar conditions to those described above, replica cultures were carried out in micro Warburg flasks. The double side-arm flasks were set up, each containing mouse ear skin floating on 2 ml Waymouth's 752/1 medium + 10% calf serum and the radioactive precursor. One of the side arms contained 0·5 ml 2N H_2SO_4. The flasks were gassed and incubated as above. After the desired culture time, the acid from the side arm was tipped into the medium. This immediately killed the tissue, and released dissolved CO_2 from the medium; 0·5 ml of 50% ethanolamine solution was injected through the rubber bung into the second side arm to adsorb the evolved CO_2. The flasks were then left overnight. The ethanolamine and the medium were separately removed from the flasks and adjusted to standard volume for radioactivity determination. It was not possible to effect the separation of the epidermis from the dermis in these cultures because, during the period of adsorption of CO_2 from the flask, the skin was damaged by exposure to the acidic medium. In these cases, therefore, the whole tissues were extracted for radioactivity.

Extraction of radioactivity from tissues.—The heat-separated epidermis and dermis were ground individually in a mortar and pestle containing 2 ml 5% (w/v) trichloroacetic acid and allowed to extract for a few hours at 4°C. After centrifugation, the supernatant was withdrawn and the residue resuspended in trichloroacetic acid. After further extraction and centrifugation, the supernatant was withdrawn. This step was repeated. The 3 acidic supernatants were combined, and stored for subsequent determination of radioactivity. The acid-extracted residues were then extracted with 5 ml chloroform-methanol (2 : 1) v/v for one hour at room temperature. After centrifugation and withdrawal of the supernatant liquids, the residue was re-extracted with chloroform-methanol twice more. To the combined chloroform-methanol extracts was added 1/5 volume of 0·05 mol/l $CaCl_2$ and the mixture vigorously mixed. After centrifugation, 2 phases were observed, an upper aqueous-methanol layer and a lower chloroform layer. The 2 phases were separated and stored for radioactivity determination. This procedure was termed Folch washing (Folch *et al.*, 1957). After chloroform-methanol extraction, the residues were dissolved in aqueous ethanolamine (50% v/v) by heating in a boiling water bath for about one hour.

The 4 extracts obtained from the cultured tissues were termed respectively *acid-soluble extract* (containing small molecular-weight precursors of macro-molecules, and intermediary metabolites); *lipid extract* (containing the different lipid classes of the skin); *Folch wash* (containing water-soluble impurities); and *tissue residue* (containing protein, polysaccharides and nucleic acids).

Thin layer chromatography.—Various lipid classes were separated by chromatography on plates of silica gel H (Merck, Camlab. Ltd., Cambridge), 250 μm thick, the developing solvent being hexane : ether : formic acid, 170 : 30 : 2 (by vol). Phospholipid types were separated on silica gel plates in the solvent system chloroform : methanol : glacial acetic acid : water, 170 : 30 : 20 : 7 (by vol). Sterol- and wax-esters were separated by the method of Haahti and Nikkari (1963), using aluminium oxide G (Merck) plates, developed in 1% benzene in hexane.

Reference lipids were identified by means of iodine vapour. Radioactivity in the separated lipids on the plates was detected by one of 2 methods: the plates were scanned for radioactivity using a thin-layer chromatogram scanner (Panax Equipment Ltd., Redhill, Surrey); or the gel of the developed plate was divided into 0·5 cm strips from the origin to the solvent front. These strips were then scraped into scintillation vials and the radioactivity determined. The first method was preferred, since the radioactive lipid could be recovered from the gel without loss, but it had the disadvantage that large levels of radioactivity (about 20,000 d/min) were necessary for accurate results. With the gel stripping method one could get a satisfactory picture of the labelling with about 2000 d/min, but the radioactive lipid could not be retrieved from the scintillation vials after counting. When the second method of radioactivity determination was used, the liquid sample was applied to the plate as a wide band (approx. 5 cm) and a 1 cm strip was taken from one edge of the developed plate for scraping off and counting.

Silicic acid column chromatography.—The radioactive lipid classes from the cultures with the 3 amino-acids leucine, isoleucine and valine were separated by column chromatography on silicic acid, as described by Prottey and Hawthorne (1966). The sample was applied to the column in pure hexane (which had been redistilled over potassium permanganate). Lipid class separation was achieved by gradient elution. Neutral lipids were eluted from the column in the sequence : sterol ester + squalene, triglyceride, sterol, as the eluant changed from 100% hexane to 100% ether. Elution with chloroform-methanol (1 : 8 v/v) removed phospholipid. Linear gradients were achieved by using a reservoir and mixer chamber of identical geometry, allowances being made for the different densities of the initial solvents in the gradient apparatus. Carrier squalene and lanosterol were added to each radioactive lipid sample to be chromatographed to aid characterization. Radioactivity was determined by counting aliquots (0·5 ml) of each 5·0 ml fraction collected.

Preparation of methyl esters.—Methyl esters of the lipid fatty acids were prepared by transmethylation. The purified lipid class (after preparative thin-layer chromatography or column chromatography) was taken to dryness under nitrogen in a test tube, and 5 ml of a mixture of methanol : benzene : conc. sulphuric acid (150 : 75 : 7·5, by vol.) was added and the mixture refluxed for 90 min at 85°C. After reaction, diethyl ether and water were added and the mixture shaken, when the methyl esters dissolved in the ether layer. The organic layer was washed with water and dried over anhydrous Na_2SO_4. Radioactive methyl ester fractions were purified further by thin-layer chromatography on silica gel H in the solvent system hexane : ether : formic acid. After addition of branched-chain fatty acid standards, analysis by gas-liquid radiochromatography was performed.

Gas-liquid chromatography.—Quantitative analysis, to calculate retention times and carbon numbers for the reference fatty acid methyl esters, was performed on a Pye 104 flame ionization detection chromatograph with a 9 ft × $\frac{1}{8}$ in. i.d. column. The stationary phase was 10% FFAP on celite support (80–100 mesh). The carrier gas was argon.

Quantitative gas-liquid radio chromatography was performed on a chromatograph first described by James and Piper (1963), as modified by James and Hitchcock (1965). Mass detection was by means of a catharometer, and radioactivity by means of a simple proportional counter. The column was a 7 ft × $\frac{1}{4}$ in. i.d. copper tube, the stationary phase being FFAP on celite.

The radioactive methyl ester mixtures to be analysed contained very little mass compared with radioactivity; thus neither the mass distribution nor the specific radioactivities of the acyl moieties of the various lipid classes could be obtained, since the catharometer was relatively insensitive. To aid identification of the radioactive peaks separated, carrier fatty acid methyl esters were added to the radioactive samples. In addition, identification of peaks by means of their " carbon numbers " (Woodford and Van Gent, 1960) was carried out. Quantitation of the radioactive peaks was achieved by triangulation.

Measurement of radioactivity.—Radioactivity was measured with a Packard Tri-carb 4322 Liquid Scintillation Spectrometer, using a dioxane-based phosphor. Efficiencies of counting were determined by using an internal standard. Radioactivity on thin-layer plates was measured as described above.

Autoradiography.—After culture in the presence of ^{14}C-sodium acetate, whole skin was rinsed extensively with ice-cold 0·9% saline solution. The tissues were fixed in formal saline prior to the cutting of frozen sections for autoradiography, as described by Rutherford and Black (1969).

RESULTS

Maintenance of Mouse Auricular Skin in vitro

It was found that samples of split-thickness mouse-ear skin were viable even after 4 days of culture at 30°C, with maintenance of normal histological morphology. Fig. 1a shows cultured mouse-ear skin after 48 hours *in vitro*. The general condition of the skin is quite good, with the continuous layer of viable basal cells being maintained in the epidermis. The nuclei of these cells appear normal. There is some oedema of the Malpighian layer, however, with intercellular separation and vacuolation. The sebaceous gland shown (SG) appears normal. Fig. 1b similarly shows 48-hour cultured mouse-ear skin, where there is more disorganization of the basal cell layer of the epidermis on the upper surface of the tissue. This figure shows an extreme cut edge of a fragment of each skin; thus the degenerative changes observed may have been due to mechanical trauma during excision, prior to culture. The figure is included, however, to demonstrate the extensive epithelial migration (marked by arrows) which had occurred from the cut edge along the underside of the tissue.

In order to confirm that, during 48 hours of culture *in vitro*, mouse-ear skin maintained normal biochemical function, the experiment illustrated in Fig. 2 was performed. Mouse-ear skin was cultured *in vitro* in the presence of ^{14}C-sodium acetate for different times. Radioactivity metabolized to CO_2, tissue lipids and to non-lipid tissue residues were measured. The most striking feature was the rapid decrease of labelled acetate in the medium during culture. The rate of uptake (i.e. metabolism) was linear for approximately 24 hours; but, after 48 hours, this rate had diminished, with the curve assuming asymptotic characteristics. At 48 hours of culture 33% of the radioactivity added to the flasks had been incorporated into the skin. Total lipid was, by far, the most highly labelled end-product of the exogenous ^{14}C-acetate, and the kinetics of lipid labelling were very similar to those for the uptake of radioactivity from the medium, i.e. linear labelling for approximately 24 hours (when 20% of the initial radioactivity had been converted to lipid), followed by a gradual tailing off of incorporation (at 48 hours 27% of the acetate had been converted to lipid). The remaining tissue radioactivity, representing the difference between that removed from the medium and that converted to lipid, was accounted for as liberated $^{14}CO_2$, presumably via Krebs Cycle oxidation (4·5% of the initial radioactivity at 48 hours), and as the non-lipid residue (1·5%), comprising labelled proteins, nucleic acids, polysaccharides and water-soluble compounds.

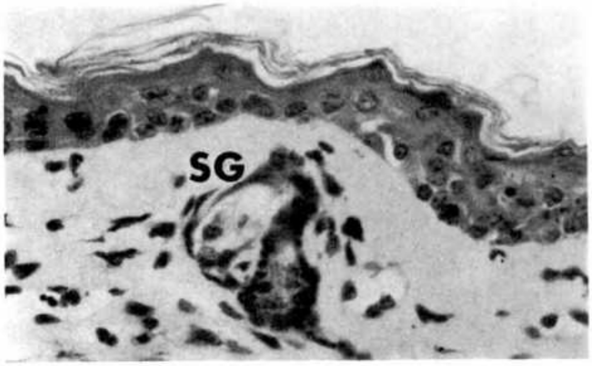

FIG. 1a.—Morphology of mouse auricular skin after culture *in vitro* for 48 hours (× 325). sg = sebaceous glands.

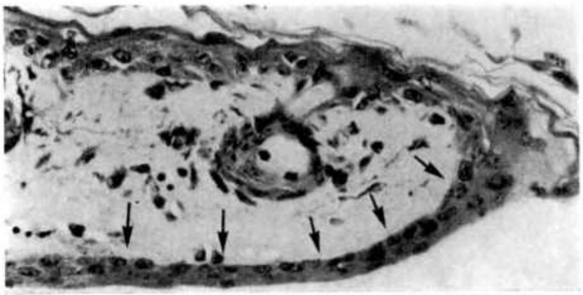

FIG. 1b.—Morphology of mouse auricular skin after culture *in vitro* for 48 hours (× 325). Arrows denote epithelial migration.

Metabolism of ^{14}Carbon-labelled Sodium Acetate and the Amino-acids Leucine, Isoleucine and Valine by Mouse-ear Skin in vitro

In order to quantify further the metabolism of the different lipid precursors under examination, mouse-ear skin was cultured in the presence of radioactive acetate, leucine, isoleucine and valine, the details of culture being given in Table I. The radioactivity incorporated was fractionated into: trichloroacetic acid-soluble extracts, lipid-soluble extracts, Folch washes of the lipid extracts, tissue residues (i.e. residual tissue radioactivity which was neither acid-soluble small molecular weight material nor lipids). Also, that radioactivity liberated as CO_2 was measured.

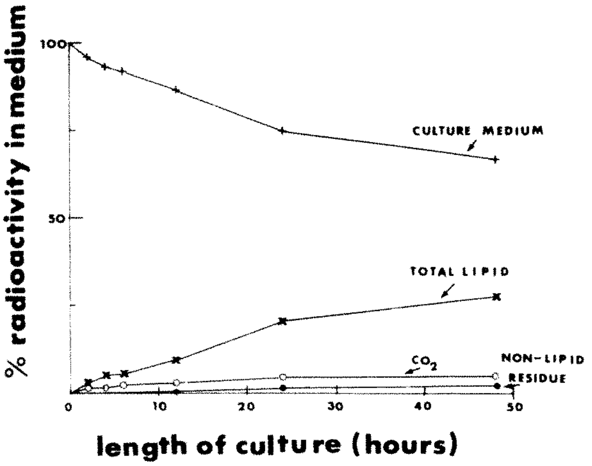

FIG. 2.—Incorporation of ^{14}C-acetate into fractions of mouse auricular skin cultured *in vitro*. (For details, see text.)

TABLE I.—*Fate of Radioactivity After Culture of Mouse-ear Skin for Various Times in the Presence of ^{14}C-labelled Precursors*

Culture time	Tissue extract	Radioactivity (mμCi)*			
		Acetate	Leucine	Isoleucine	Valine
5 hours	Trichloroacetic acid soluble	46·4	24·6	26·0	17·8
	Lipid soluble	531·4	17·2	13·9	1·2
	Folch washes	7·9	4·7	2·6	2·1
	Tissue residues	30·0	77·8	33·9	9·8
	CO_2	125·3	42·3	61·6	9·2
25 hours	Trichloroacetic acid soluble	45·4	21·2	23·9	13·8
	Lipid soluble	1458·7	92·6	54·9	6·4
	Folch washes	19·0	16·0	15·8	5·0
	Tissue residues	88·2	749·0	588·2	185·1
	CO_2	385·1	170·7	190·1	179·0

* The values represent the mean of 2 experiments. Each flask contained the skin from 4 mouse-ears in the presence of 5 μCi of radioactivity.

Table I shows that, during a 5-hour culture, the trichloroacetic acid-soluble and the tissue residue radioactivities were, for all 4 precursors studied, quite low and of roughly equal orders of magnitude. Similarly, the Folch washes were equivalent and very low indeed. The lipid radioactivities were totally different, however, since the radioactive lipid with ^{14}C-acetate as the precursor was massive (10% of the added level), whereas, with both leucine and isoleucine, the lipid radioactivity was lower than that of their respective tissue residues. Lipid labelling from ^{14}C-valine was insignificant at 5 hours. Similarly, with acetate as the precursor, far more $^{14}CO_2$ was released than with the 3 amino-acids.

After 25 hours of culture the pattern of labelling was even more complex. In every case the trichloroacetic acid-soluble fractions were unchanged (or slightly lower) compared with their 5-hour values. This suggested that steady-state incorporation had been reached of the precursors in the medium into the tissue pools available for further metabolism. This was not seen in the lipids of the 25-hour cultures, however, where, for each precursor examined, the radioactivity was between 3 and 5 times that of the 5-hour level. Again, the acetate cultures contained far more lipid-soluble radioactivity compared with the 3 amino-acids, a trend reflected in the $^{14}CO_2$ levels also. The tissue residue radioactivities were grossly different, where each of the 3 amino-acids were extensively incorporated into the tissue residues (this being due, presumably, to protein synthesis); however, with ^{14}C-acetate tissue residue, radioactivity was relatively low. Folch wash radioactivities were consistently very low for all precursors studied. Two main trends were apparent from this experiment, namely, that sodium acetate was preferentially converted to lipid compared with the amino-acids which metabolized to protein, and that leucine and isoleucine showed overall similar patterns of radioactivity distribution which were in almost every case greater than the utilization of valine.

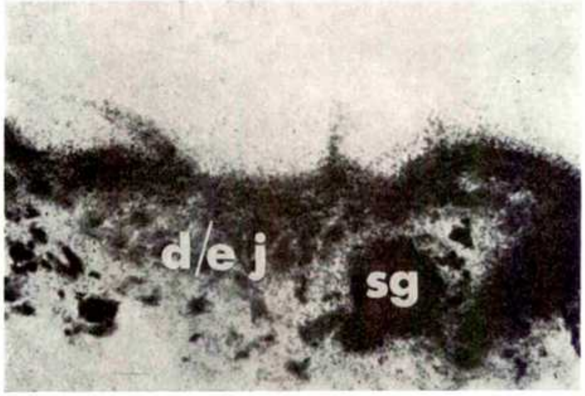

FIG. 3.—Autoradiogram of mouse auricular skin cultured *in vitro* in the presence of ^{14}C-acetate for 48 hours (× 325).

Autoradiographic Localization of the Site of Lipogenesis in Cultured Mouse-ear Skin

Fig. 2 demonstrates the facility with which exogenous ^{14}C-acetate was incorporated into cutaneous lipids. Mouse-ear skin, which had been cultured under identical conditions to those of Fig. 2, was processed for autoradiography, and this is shown in Fig. 3. The definition of the tissue, which was stained with H. and E., is not very clear, but the majority of the tissue lipid radioactivity (shown in the figure as silver grain blackening) was localized in the sebaceous glands (SG). Although the radioactivity in the rest of the dermal matrix was very sparse, the whole of the epidermis, i.e. that region above the dermo-epidermal junction (D/EJ), contained diffuse radioactivity. The figure suggests that lipid synthesis from ^{14}C-acetate in split-thickness mouse-ear skin was localized in 2 main regions, namely, in the sebaceous glands, buried within the dermis, and in the epidermis.

Lipid Synthesis in Mouse-ear Epidermis and Dermis

In a similar experiment to that described above mouse-ear skin was cultured *in vitro* in the presence of radioactive sodium acetate, leucine, isoleucine and valine, and the epidermis was separated from the underlying dermis by heat-treatment. The isolated epidermis and dermis samples were then serially extracted with trichloroacetic acid, chloroform-methanol and 50% ethanolamine as before, and the radioactivities recorded in Table II. This table corroborates the findings of Fig. 3 that lipid production from ^{14}C-acetate was more active in the dermis (i.e. the sebaceous glands, which remain in the dermis after heat-separation of the epidermis) than in the epidermis. Similarly, the findings of Table I, that leucine and isoleucine were metabolized to specific tissue components at roughly equivalent rates, but much less effectively than acetate for lipids, and far more effectively than valine into proteins, were also seen.

TABLE II.—*Fate of Radioactivity in Separated Epidermis and Dermis of Mouse-ear Skin After Culture in the Presence of ^{14}C-labelled Precursors*

		Radioactivity (mμCi)			
Culture time	Tissue extract	Acetate	Leucine	Isoleucine	Valine
5 hours	Epidermis trichloroacetic acid	8·0	4·0	8·9	5·4
	Epidermis lipid	48·3	3·6	4·7	0·6
	Epidermis Folch wash	0·8	1·2	2·8	0·6
	Epidermis tissue residue	6·4	7·6	19·2	1·5
	Dermis trichloroacetic acid	41·0	14·7	27·9	14·8
	Dermis lipid	451·8	13·8	13·1	0·9
	Dermis Folch wash	6·8	1·6	4·2	1·3
	Dermis tissue residue	20·0	62·0	70·4	9·7
25 hours	Epidermis trichloroacetic acid	12·0	6·4	8·2	11·3
	Epidermis lipid	169·1	31·0	13·7	2·7
	Epidermis Folch wash	8·1	4·2	5·7	3·0
	Epidermis tissue residue	15·3	218·0	71·6	43·3
	Dermis trichloroacetic acid	76·4	20·5	29·2	14·0
	Dermis lipid	1345·8	83·1	54·1	7·1
	Dermis Folch wash	14·4	22·2	22·1	3·9
	Dermis tissue residue	64·4	358·6	183·2	70·9

Conditions as for Table I, but tissues heat separated after culture.

As seen in Table II, the amount of labelled lipid formed in the cultured skin from ^{14}C-acetate was very high: in the epidermis after 25 hours of culture 169 mμCi of radioactivity, or nearly 3·5% of the initial level added to the cultures; in the dermis 1345 mμCi, or 27% of the initial level, were metabolized to lipid. For the 3 amino-acids studied, these levels were very much lower: for valine, in the epidermis after 25 hours culture 2·7 mμCi, or about 0·05%, and in the dermis 7·1 mμCi, or about 0·14%, was found in lipid. Such low levels impose obvious constraints upon the amount of radioactive lipid available for detailed analysis; thus larger scale cultures were performed in order to prepare adequate radioactive products.

Identification of the Lipid Classes Labelled in Mouse-ear Epidermis and Dermis When Cultured in the Presence of Radioactive Acetate, Leucine, Isoleucine and Valine

In order to investigate the precise nature of the radioactive lipids synthesized during culture of mouse-ear skin in the presence of radioactive sodium acetate, leucine, isoleucine and valine, the following larger scale cultures were performed.

Two culture flasks were used for each radioactive precursor under examination, each flask containing the skin from 4 mouse-ears, floating on 2 ml Waymouth's

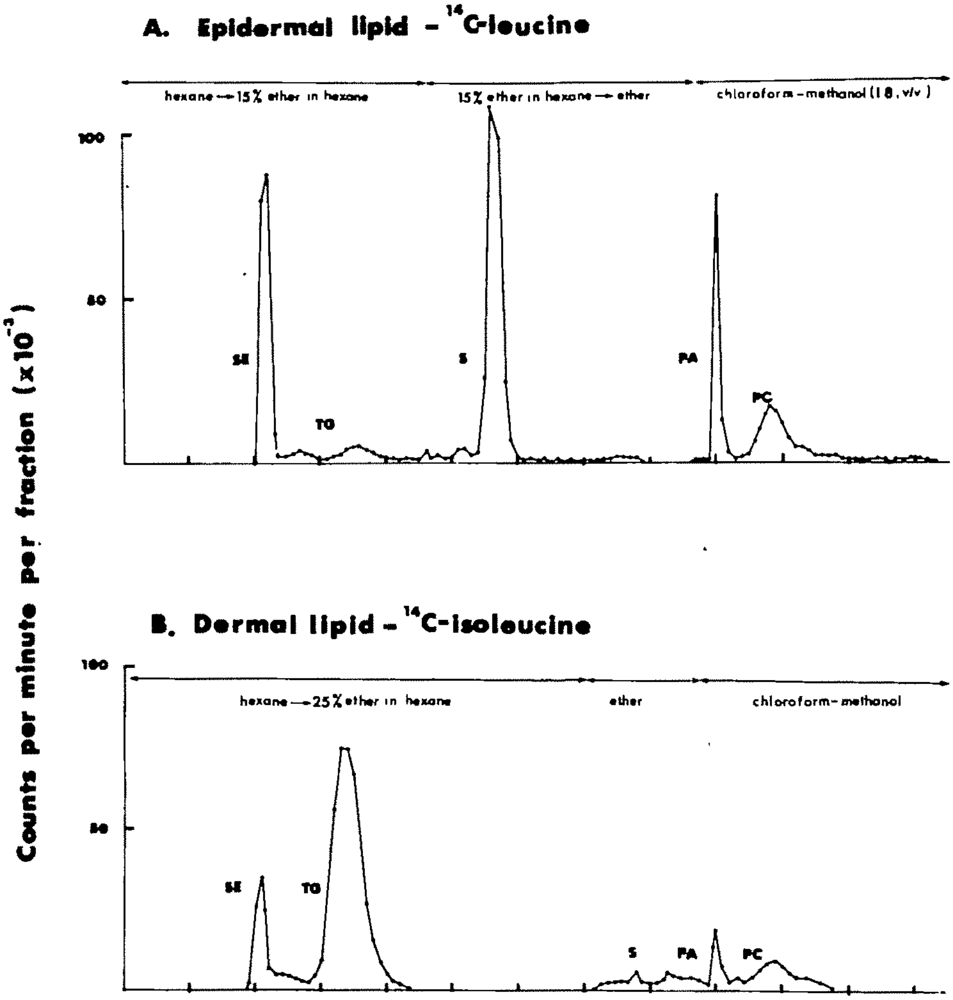

FIG. 4.—Silicic acid column chromatographic separation of ^{14}C-labelled lipids from culture *in vitro* in the presence of radioactive precursors. A. Total epidermal lipid from ^{14}C-leucine culture. B. Total dermal lipid from ^{14}C-isoleucine culture. (For other details, see text.)

752/1 medium + 10% calf serum. Twenty-five μCi of the respective labelled substrate was added to each flask, which was then maintained at 30°C for 48 hours, when the epidermis and dermis were separated by heat-treatment. The tissues from each of the duplicate cultures were combined, and the amounts of radioactivity therein converted to lipid, shown in Table III.

Table III illustrates again the features already seen, namely, that acetate was by far the most effective precursor of lipid; that leucine, isoleucine and valine (in that order) were much less effectively converted to lipid (by an order of magnitude) than acetate; and that, in every case, the sebaceous glands within the dermis were far more active than epidermis.

The radioactive lipids were fractionated into individual lipid classes by chromatography on columns of silicic acid, using linear gradient elutions. Example of 2 such elutions is shown in Fig. 4.

The discrete peaks of radioactivity for each of the 8 radioactive lipid extracts (that is, epidermal and dermal lipid extracts from the acetate, leucine, isoleucine and valine cultures) were pooled, positively identified by thin-layer chromatography using standard lipids, and the total patterns of the radioactivity distribution within the various lipid classes calculated. These are shown in Table IV.

With each precursor tested, phosphatidylcholine and phosphatidic acid were the principal labelled phospholipids. With ^{14}C-acetate, labelling of phosphatidylethanolamine was also evident, especially in the dermal products, but no labelling was detected in this lipid or in lysophosphatidylcholine or sphingomyelin, when the substrate was any one of the 3 amino-acids. This failure of detection was probably due to the much lower levels of radioactive lipids obtained from the labelled amino-acid cultures (Table III). With ^{14}C-acetate, in both the epidermis and the dermis, labelled phospholipid accounted for 20% of the total lipid products. With ^{14}C-leucine and ^{14}C-isoleucine, both showed more phospholipid labelling in the epidermis (about 25% of the total) than in the dermis (13%). For ^{14}C-valine, phospholipid labelling in both the epidermis and dermis was low (8%). Labelled sterol was consistently higher in the epidermis than the dermis with ^{14}C-acetate, leucine and isoleucine. With valine, about 9% was detected in the dermis only, none in the epidermis. The value of 38·2% epidermal sterol radioactivity from ^{14}C-leucine was remarkably high, this probably being due to the fact that leucine may be degraded to 3-hydroxy-3-methyl glutaryl CoA, which is a specific precursor of sterols. Very small levels of radioactive free fatty acids were detected, and only when ^{14}C-acetate was the precursor. Labelled trigly-

TABLE III.—*Large-scale Preparation of Labelled Lipids by Culturing Skin* in vitro *with ^{14}C-acetate, Leucine, Isoleucine and Valine for* 48 *Hours*

	Percentage of added radioactivity incorporated into lipid	
Precursor	Epidermis	Dermis
2-^{14}C-acetate	7·6	42·7
(U)-^{14}C-leucine	0·94	2·1
(U)-^{14}C-isoleucine	0·34	1·06
(U)-^{14}C-valine	0·11	0·23

For each radioactive precursor studied 2 culture flasks were used, each containing skin from 4 mouse-ears in the presence of 25 μCi radioactivity. Culture was for 48 hours at 30°C.

ceride followed a distinct and noteworthy pattern. For each of the 4 precursors studied there was more radioactive triglyceride in the dermis than the epidermis. With leucine and isoleucine as substrates, this level was about 60%. Also, a trend was seen with labelled sterol- and wax-esters where, for each precursor studied, more radioactivity in these lipids was detected in the epidermis than the dermis. With ^{14}C-valine, in both epidermis and dermis, labelled sterol- and wax-esters contained the greatest part of the incorporated radioactivity.

The chromatographic system used for separating the different lipid classes described in Table IV caused sterol esters, wax esters and squalene to move with the same mobility. Squalene could be separated from this mixture by a further development of the thin-layer plate in the pure hexane; however, no significant radioactivity was seen in this lipid after 48 hours *in vitro*. In preliminary studies, using ^{14}C-acetate, labelled squalene was detected, but never at levels of more than a few per cent of the total lipid radioactivity. Sterol esters and wax esters are very similar in polarity, and, consequently, were found to be inseparable when using silicic acid chromatographic systems. However, using the aluminium oxide system of Haahti and Nikkari (1963), a separation was achieved, when it was found that sterol esters contained most of the radioactivity, which was mainly in the acyl moiety, with only traces in the sterol nucleus.

TABLE IV.—*Lipid Classes Labelled in Mouse-ear Skin Cultured in the Presence of ^{14}C-sodium Acetate, Leucine, Isoleucine and Valine*

	Proportion of total lipid radioactivity (%)							
	Acetate		Leucine		Isoleucine		Valine	
Lipid class	Epidermis	Dermis	Epidermis	Dermis	Epidermis	Dermis	Epidermis	Dermis
Lysolecithin	0·2	0·3	—	—	—	—	—	—
Sphyngomyelin	1·7	0·6	—	—	—	—	—	—
Phosphatidylcholine	7·6	6·6	20·1	7·9	11·3	8·6	3·9	5·4
Phosphatidylethanolamine	2·7	9·6	—	—	—	—	—	—
Phosphatidic acid	7·1	4·0	6·2	4·6	12·4	4·5	4·2	1·8
Total phospholipid	19·3	21·1	26·3	12·5	23·7	13·1	8·1	7·2
Sterol	16·6	12·2	38·2	12·1	19·7	7·0	0	8·9
Free fatty acid	1·8	0·7	—	—	—	—	—	—
Triglyceride	13·9	38·8	13·0	59·2	—	67·1	6·2	12·3
Sterol/wax esters	48·4	26·6	22·5	16·2	44·4	12·8	85·7	71·6
Unidentified	—	—	—	—	12·2	—	—	—

The skin from 4 mouse-ears was cultured in the presence of 25 μCi of the requisite radioactive tracer for 48 hours and in duplicate. The flasks were combined and the epidermis separated from the dermis. Lipids were chromatographed on columns of silicic acid.

Patterns of Fatty Acids Synthesized from ^{14}C-acetate, Leucine, Isoleucine and Valine

The saponifiable lipids labelled during culture in the presence of the various precursors were transmethylated, and the radioactive methyl esters obtained were analysed by gas-liquid radiochromatography. In some cases, due to insufficient radioactivity, certain lipid types were not analysed, or were combined (e.g. total phospholipid for the 3 amino-acid cultures). Radioactive fatty acid methyl esters were identified with reference compounds by means of the specific " carbon-number ". Table V lists the pattern of radioactivity obtained. The gas-liquid

TABLE V.—*Labelled Fatty Acid Patterns of Lipids Synthesized in Mouse-ear Epidermis and Dermis During Culture in vitro in the Presence of ^{14}C-labelled Acetate, Leucine, Isoleucine and Valine*

			% of total radioactive fatty acid methylesters recovered from column																			
			2-^{14}C-Sodium acetate								^{14}C-(U)-leucine					^{14}C-(U)-isoleucine					^{14}C-(U)-valine		
		Carbon	Epidermis (E)						Dermis (D)		E		D			E		D			E	D	
Fatty acid		no.	SE	TG	FFA	PC	PE	PA	SE	TG	SE	PL	SE	TG	PL	SE	PL	SE	TG	PL	SE	SE	TG
	12 : 0	12·0	trace	—	—	—	—	—	0·3	0·2	—	—	—	—	—	—	—	—	—	—	—	—	—
	12 : 1	12·2	1·2	—	—	—	—	—	0·6	—	—	—	—	—	—	—	—	—	—	—	—	—	—
iso-	14 : 0	13·5	2·3	—	—	—	—	—	0·7	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	14 : 0	14·0	1·2	4·2	3·8	3·0	trace	3·1	0·9	7·4	18·2	6·2	2·1	7·5	3·9	—	7·0	11·7	5·3	2·8	—	—	—
	14 : 1	14·2	10·9	1·4	trace	2·3	trace	trace	6·2	0·5	—	—	—	1·0	2·0	—	3·3	—	0·8	1·0	—	—	—
iso-	15 : 0	14·5	—	—	—	—	—	—	—	—	16·6	1·0	5·3	0·4	—	—	—	—	—	—	—	—	—
anteiso	15 : 0	14·65	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0·6	trace	—	—	—
	15 : 0	15·0	—	—	—	—	—	—	—	—	—	2·0	4·3	0·4	—	—	—	—	0·9	2·9	—	—	—
iso-	16 : 0	15·5	4·3	—	—	—	—	—	3·4	—	—	—	4·6	—	—	—	—	—	—	—	100·0	85·7	14·8
	16 : 0	16·0	5·1	20·3	26·7	29·1	16·2	24·5	3·6	26·4	13·2	40·0	19·8	29·8	38·0	trace	27·8	54·5	33·6	30·1	—	—	32·1
	16 : 1	16·2	18·4	24·7	21·4	17·0	10·6	12·2	14·4	25·6	trace	9·0	11·8	18·5	10·0	—	12·2	33·8	14·5	7·8	—	—	10·5
iso-	17 : 0	16·5	—	—	—	—	—	—	—	—	18·2	3·0	16·1	1·9	7·0	—	—	—	—	—	—	—	—
anteiso	17 : 0	16·65	—	—	—	—	—	—	—	—	—	—	—	—	—	trace	—	—	1·1	trace	—	—	—
	17 : 0	17·0	1·6	—	—	—	—	—	1·5	—	—	—	—	—	—	—	—	—	—	2·7	—	—	—
	17 : 1	17·2	1·4	—	—	—	—	—	0·5	—	—	—	—	—	—	—	—	—	—	—	—	—	—
iso-	18 : 0	17·5	11·6	—	—	—	—	—	9·1	—	—	—	—	—	—	—	—	—	—	—	—	14·3	—
	18 : 0	18·0	8·3	14·3	22·1	21·1	26·2	23·9	9·3	13·9	18·0	28·2	19·9	14·3	19·1	trace	34·5	trace	22·8	30·8	—	—	24·2
	18 : 1	18·2	11·7	28·5	26·1	25·3	46·9	29·6	14·9	25·9	15·9	9·1	16·1	26·1	20·0	—	15·2	trace	20·2	21·9	—	—	18·4
	18 : 2	18·6	1·2	—	—	—	—	—	trace	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	18 : 3	19·2	3·4	—	—	—	—	—	1·8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
iso-	20 : 0	19·5	7·6	—	—	—	—	—	8·3	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	20 : 0	20·0	2·7	5·3	trace	2·2	trace	6·7	9·0	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	20 : 1	20·2	7·3	1·7	trace	trace	trace	trace	10·9	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Abbreviations: SE, sterol esters; TG, triglycerides; FFA, free fatty acids; PL, total phospholipids; PC, phosphatidylcholine; PA, phosphatidic acid.

radiochromatograph used was not sufficiently sensitive to record the masses of each respective radioactive fatty acid; thus specific activities could not be determined.

Several general features are seen in Table V. For each particular labelled substrate used the pattern of radioactive fatty acids esterified in each individual lipid class was similar in both epidermis and dermis. Also, it may be seen that the sterol esters are, in every instance, different from the other lipid classes by virtue of their rather complex labelling patterns. The sterol esters contained more uncommon labelled fatty acids, especially when compared with triglycerides. In the ^{14}C-acetate cultures both dermal and epidermal triglycerides contained mainly saturated and unsaturated even-numbered straight chain fatty acids. The sterol esters, in addition to these acids, contained also odd-numbered chain fatty acids and even- and odd-numbered isobranched fatty acids.

Since it was anticipated that branched-chain fatty acids would be synthesized during culture, the specific precursors of certain fatty acid structures were used. Leucine was included as a precursor of odd-numbered chain iso-branched acids, isoleucine for odd-numbered chain isoacids. To a certain degree these specific precursors produced the predicted result. In the ^{14}C-leucine cultures, iso-pentadecanoate (iso-C15 : 0) and iso-heptadecanoate (iso-C17 : 0) were found in all lipid classes of both epidermis and dermis, with the sterol esters containing the highest proportions. With ^{14}C-valine, iso-palmitate (iso-C16 : 0) was the sole product in the epidermis where this acid was found almost exclusively in sterol esters (see Table IV). In the dermal sterol esters, a small amount of iso-stearate (iso-C18 : 0) was found as well as iso-palmitate. With isoleucine, however, only traces of anteiso-pentadecanoate (anteiso-C15 : 0) and anteiso-heptadecanoate (anteiso-C17 : 0) were formed, in quantities too small to measure accurately. These acids were found only in dermal phospholipid and epidermal sterol esters.

In addition to the synthesis of specific branched fatty acids from the 3 radioactive amino-acids, normal even-numbered saturated and unsaturated radioactive fatty acids were detected, indicating that degradation of the amino-acids to acetate had occurred. Thus the labelling patterns obtained using these precursors were complicated due to products of the specific incorporation of branched structures into branched fatty acids, and the incorporation of acetate units into straight-chain fatty acids.

It could be seen, however, that the results obtained from the ^{14}C-acetate tracer studies were corroborated by the amino-acid studies. Odd-numbered chain iso-acids were not detected using ^{14}C-acetate; and, when their specific precursor was used (leucine), these acids were always minor products. Odd-numbered chain anteiso acids were similarly not seen when ^{14}C-acetate was the precursor, and accordingly only minute traces were observed in the ^{14}C-isoleucine cultures. Even-numbered chain iso-acids, however, were important products of the ^{14}C-acetate cultures, epidermal sterol esters containing a total of 25·8%, and dermal sterol esters 21·5% isobranched acids of the series C14 : 0, C16 : 0, C18 : 0 and C20 : 0. Similarly, in the epidermal and dermal sterol esters of the ^{14}C-valine cultures, even-numbers chain iso-acids were the sole products.

The gas-liquid radio chromatography was performed under isothermal conditions at 220°C when fatty acid methyl esters with chain lengths greater than C20 had very long retention times. In order to ensure that no radioactivity was associated with such compounds, each chromatogram was continued for up to

2 hours after the elution of C20 : 0, but radioactivity in fatty acids of greater chain length than C20 : 0 was never seen.

DISCUSSION

Viability of Mouse-ear skin in Culture

The method of culturing mouse-ear skin *in vitro* was essentially that described by Gillette and Findley (1958), which was very similar to the original report of Medawar (1948) on the floating culture technique. Histological examination of the skin after two days *in vitro* (Fig. 1b) showed that epithelial migration (epiboly) had occurred around the cut edges. Preliminary studies had already revealed that the tissues could be maintained *in vitro*, with preservation of normal morphology and extensive epiboly for up to 5 days, if the temperature of incubation was 30°C. This was not possible at 37°C, however, where rapid tissue degeneration occurred. The findings in Fig. 2, that ^{14}C-acetate was still being taken up from the medium into cultured slices and converted to lipid at 48 hours of culture, would corroborate that the tissues *in vitro* could still perform complex metabolic reactions. The observed fall-off in the rate of acetate uptake at 48 hours *in vitro* was probably due either to depletion of the precursor in the medium, or to dilution of the labelled acetate by metabolism of the glucose in the medium by the skin. This would not necessarily indicate a decreased rate of lipid turnover, however. Similar experiments with mouse-ear skin in culture, using other radioactive precursors, showed the following conversions of radioactivity in the media to tissue lipid, at 24 hours of culture: ^{32}P-orthophosphate, 0·1%; ^{14}C-glycerol, $< 4\%$; ^{14}C-mevalonic acid, 1% (unpublished data). The much greater incorporation of acetate at 24 hours (about 20%, from Fig. 2) reflected the facility of uptake of this molecule as substrate for a lipogenesis.

There is a wealth of published data on lipid production from radioactive acetate by skin *in vitro*, but little has been reported on cultures for extended periods of incubation. Nicolaides and Rothman (1955) cultured scalp and forearm skin with ^{14}C-acetate for 7 hours; Griesemer and Thomas (1963) for 1 hour and 3 hours; and Vroman *et al.* (1969) for 6 hours. These data indicate that, during culture times of at least 2 days, lipogenesis still continued in the skin.

Separation of Epidermis and Dermis after Culture

In this study the epidermis and dermis were separated by brief heat treatment (Baumberger *et al.*, 1942), a method which is particularly effective for relatively hairless skin. For mouse-ear skin, 90 seconds at 56° C was sufficient to effect the separation. Histological examination of the fragments showed the point of separation to be the dermo-epidermal junction, with the sebaceous glands remaining within the dermis. There were obvious signs of tissue damage, dehydration and cell death arising from the heat treatment, but it was assumed that there was no change in the radioactivity already incorporated into the tissues. Preliminary experiments have been performed on the viability in culture of heat-separated epidermis. When this was cultured for 24 hours under conditions described for Fig. 2, the amount of lipid radioactivity was only 0·3% of that added to the medium, compared with about 20% in untreated whole skin. Baumberger *et al.* (1942) reported that respiration in heat-separated epidermis was only 25% of that shown by mechanically-separated tissues. Similarly, Lawrence and Ricketts

(1957) described how exposure of skin to heat prior to culture severely affected the tissues' ability to incorporate radioactive precursors. The heat-separation method would therefore be of no value in the preparation of dermis-free epidermis for subsequent culture. However, by using this technique after culture in the present studies, a rough differentiation between epidermal and dermal metabolism has been possible. It is known that certain lipids (in particular those containing polyenoic acids) are susceptible to oxidation in the presence of air (Rouser *et al.*, 1967). During the heat-separation of skin, similar oxidation of radioactively-labelled fatty acids could have occurred also. For this reason the tissues were always covered by droplets of saline during heating to exclude air, and thus reduce autoxidation. The subsequent gas-liquid chromatograms showed no evidence of oxidation products of these labelled fatty acids.

Sites of Lipogenesis in Mouse-ear Skin

In the present study a distinction was made between epidermis and dermis after heat-separation of the tissue. By considering Fig. 3, it was assumed that labelling in the separated dermis was due to sebaceous gland activity, whereas that in the epidermis was due to epidermal cell turnover. Fig. 3 is an autoradiograph of mouse-ear whole skin cultured for 48 hours in the presence of 2·5 μCi 2-^{14}C-sodium acetate. Whereas the tissue did not stain deeply, and high silver grain intensities tended to obliterate certain morphological structures, it could be seen that the silver grain density (which, as shown in Fig. 2 is due to lipid labelling almost entirely) was low in the dermal matrix (DM), whereas in the sebaceous glands (SG) there was massive lipid labelling. The dermo-epidermal junction (D/EJ) represents the point of separation after heat-treatment of the tissue, and quite uniform silver grain intensities throughout the whole of the epidermis could be seen. Although the stratum corneum was not clearly defined, there was evidence of lipid labelling throughout this region too.

It was assumed, also, that no radioactive sebum, synthesized during culture *in vitro* in the sebaceous glands, was secreted on to the skin surface, for the following reasons. After a routine culture for 48 hours with ^{14}C-acetate, the surface of the skin fragments was swabbed with cotton wool soaked in hexane, in order to remove surface lipid. No appreciable labelled lipid was detected in these swabbings. In addition, no significant quantities of radioactive free fatty acids were found in the epidermal lipid fractions for any of the precursors studied. It is generally believed that sebaceous gland triglycerides are hydrolysed to free fatty acids in the lumen of the sebaceous glands during secretion (Nicolaides and Wells, 1957).

It can be seen from Tables II and IV that the labelled lipid patterns for each precursor used were quite complex, with specific differences between epidermis and dermis, and with numerous different lipid classes exhibiting turnover. Nicolaides and Rothman (1955) suggested that human epidermis was the site of sterol synthesis, whereas squalene was formed in the sebaceous glands. This idea was corroborated by Griesemer and Thomas (1963), who showed that skin with greater densities of sebaceous glands synthesized more radioactive squalene from ^{14}C-acetate than skin with few glands. Similarly, Gaylor (1963) showed that the metabolism of ^{14}C-acetate to squalene and free sterols had essential differences in rat skin epidermis and sebaceous glands. Chemical studies have also revealed specific differences in the lipids of the epidermis and the sebaceous glands, or the surface fat which is derived mainly from sebaceous secretion. Reinertson and Wheatley (1959) reported that the Malpighian layer of human skin contained more sterol and phospholipid than the sebaceous glands, which were relatively enriched in squalene, wax alcohols and polyhydroxy compounds. Mustakallio *et al.* (1964) confirmed these observations.

Sterol metabolism has been thought to differ between the sebaceous glands and epidermis. Wilson (1963) suggested that, in the sebaceous glands, sterols were synthesized via the Kandutsch–Russell (1960) pathway, where the intermediates in the conversion of lanosterol to cholesterol have saturated side chains and are in the esterified form (e.g. $\Delta7$ cholesterol and $\Delta5$, 7 cholestadienol), whereas, in the epidermis, sterol is synthesized via the Bloch (1965) pathway, where all the nuclear transformations (demethylations at Carbons 4 and 14, and the double bond shift from C7 to C5) precede the final reduction of $\Delta5$, 24 cholestadienol to cholesterol. Such theories have thrived on the many reports of differences between the sterol pattern of epidermis and of sebaceous glands, many of which are equivocal. However, there is no reason for assuming that lipogenesis in these 2 morphologically distinct regions should be similar, since there are clear distinctions in function.

We have found in mouse-ear that the epidermis produced comparatively more phospholipid, sterol and sterol esters than the dermis, where the predominantly labelled lipid was triglyceride. Epidermal lipid serves 2 main functions, namely, as a source of energy for cellular reactions and as building blocks for cellular architecture. In the basal layer an epidermal cell will divide, and sterols and phospholipids are required for the new membranes and organelles produced. These lipids are made from glucose, acetate and amino-acids which are derived from the dermal vascular system. After maturation in the basal region of the epidermis the daughter cells differentiate into keratinocytes and, during their transformation into squamous material, there is denucleation, complete disruption of cellular organization and breakdown of constituent cell membranes. Those lipids which are released from membranes during the formation of keratin serve as substrates for energy-requiring processes (Cruickshank *et al.*, 1962). This is probably necessary because the concentration of free glucose in the upper levels of the epidermis is very low (Long, 1969). The sterol esters of epidermis have been re-appraised by Yardley (1969) in the light of previous information, and he suggests that, during the process of normal keratinization, the epidermis elaborates a lipoprotein containing a particular species of sterol ester. These lipids, which have been reported in high concentrations in the stratum corneum (Kooyman, 1932), may be essential to the ordered structure of keratin fibrils (Swanbeck and Thyresson, 1962). Because free cholesterol is detected only in cell membranes and because the process of keratinization involves the differentiation and destruction of basal cell membranes, it is argued that the sterol liberated is esterified to fatty acid (from phospholipid) to form sterol esters, possibly by a mechanism similar to that described by Glomset (1962). Gara *et al.* (1964) and Freinkel and Aso (1969) have described enzymic esterification of sterol in the epidermis.

Fig. 3 and Table IV indicated that the sebaceous glands were very active in synthesizing triglyceride from all the precursors studied except valine. This suggested, therefore, that triglyceride was a principal component of sebum in the mouse (Nicolaides *et al.*, 1968).

Types of Lipids Synthesized During Culture of Mouse-ear Skin in vitro

The pattern of labelling in mouse-ear lipids bears some relationship to the data of Vroman *et al.* (1969), who showed that, in human preputial and abdominal skin cultured *in vitro* with ^{14}C-acetate, the distribution of radioactivity in the lipids was phospholipids > free sterol > triglycerides > squalene. In this case, however, the cultures were for 6 hours, and no fractionation into epidermal and dermal radioactivity was made. The lipid classes labelled in the amino-acid cultures showed differences, however, compared with the data reported by Wheatley *et al.* (1967), who perfused isolated dog skin with radioactive precursors and showed that, with ^{14}C-leucine and ^{14}C-isoleucine, triglyceride was generally the most abundant labelled lipid, with phospholipid and sterol labelling never greater than about 12%. When ^{14}C-valine was perfused, methyl esters and free fatty acids (both probably artefacts) contained greater than 50% of the radioactivity. Again, no attempt was made to distinguish dermal from epidermal lipogenesis, but they did report that the "efficiency of lipogenesis", relative to ^{14}C-acetate, of ^{14}C-leucine, isoleucine and valine was, respectively, 14%, 12·6% and 8·4%. From Table I, after 25 hours of culture, we found these values, relative to the incorporation of ^{14}C-acetate into lipid, to be, for leucine 6·3%, for isoleucine 3·7% and for valine 0·4%. Wheatley suggested that the differences in labelling the various lipid classes from amino-acids was due to the tissues' requirement for the specific fatty acid, and that each amino-acid was the precursor. Thus, since the incorporation of valine was very low, the isopalmitate and isostearate which were derived from this amino-acid were not common constituents of the lipids. We have found this statement to be false, however. Both ^{14}C-leucine and ^{14}C-isoleucine were degraded to ^{14}C-acetate and then incorporated into normal even-numbered chain fatty acids preferentially, rather than being converted exclusively to odd-numbered iso- and odd-numbered anteiso acids respectively. With valine, however, this was preferentially converted to its specific even-numbered iso-acids. Hence the utilization of leucine and isoleucine was not solely because of branched fatty acid synthesis.

The gas-liquid radiochromatograph used in this work was too insensitive to give mass patterns of the constituent fatty acids of mouse lipids after culture; however, the data reported by Carruthers have been compared with the data of Table V. Carruthers (1962) reported the fatty acid patterns of triglycerides of mouse epidermis and dermis to be quite similar. Oleic acid was the principal component (56%), with palmitic acid constituting 20% of the total fatty acids. The acids C16 : 1, C18 : 0 and C18 : 2 were respectively 6%, 4% and 10%. In the present study, in triglycerides of both epidermis and dermis, synthesized from all 4 radioactive precursors, saturated and monosaturated C16 and C18 acids were predominant (Table V). For phospholipids of the dermis, Carruthers found that palmitic acid comprised 47%, with C18 : 1 and C18 : 0 much less abundant, being 17% and 14% respectively. Epidermal phospholipids had more complex fatty acid patterns, however, containing many different types. Palmitic acid represented only 17% of the total, with 25% oleate and 10% stearate. There were also traces of C20 : 0, C22 : 0 and C26 : 0 acids, with C24 : 0 (lignoceric acid) comprising 12% of the total. Wilkinson and Karasek (1966) later confirmed that lignoceric acid and cerotic acid (C26 : 0) were principal components of albino mouse skin lipids, especially the epidermal phospholipids.

In addition, they described a series of long-chain monoenoic fatty acids from

27 to 36 carbon chains, which comprised some 20% of the total epidermal fatty acids. In the present study, however, such changes were not so marked. Epidermal phospholipid fatty acids were very similar to those in dermal phospholipids. With ^{14}C-acetate as the precursor, oleate was always more highly labelled than stearate or palmitate; however, with the 3 amino-acids, generally, the saturated fatty acids were more highly labelled than their monoene counterparts. Carruthers (1964) also studied the combined wax- and sterol esters of mouse epidermis, and found a pattern of constituent fatty acids which differed greatly from the other lipid classes. In this fraction C18 : 1 was the predominant fatty acid (26%), although the levels of C16 : 1 (10%), C20 : 1 (12%) and C22 : 1 (12%) were significant. The wax- and sterol- esters were unlike other lipid classes in that they contained iso-branched acids, including isopalmitate, isostearate, isoarachidate and isobehenate, as well as the anteiso-branched forms of C15 : 0, C17 : 0, C19 : 0 and C21 : 0. Odd-numbered chain normal fatty acids of these were also present. The total percentage of even-numbered chain iso-acids was about 10%, of odd-numbered chain anteiso acids about 8%, and of odd-numbered chain normal acids about 4%. In a similar fashion to the mass patterns reported by Carruthers (1964), we have found that even-numbered chain iso-acids were important members of both epidermal and dermal sterol esters, when both ^{14}C-acetate and ^{14}C-valine were the labelled lipid precursors; however, the presence of labelled anteiso or normal chain odd-numbered fatty acids were not consistently noted.

The fatty acid radioactivity patterns of respective lipid classes were generally similar, no matter which radioactive precursor was used, and we were unable to state whether the data shown in Table V represented complete *de novo* synthesis of fatty acids, or merely chain elongation of pre-existing unlabelled intermediates with ^{14}C-acetate units from the degradation of the labelled amino-acids. Wilkinson (1970a) showed, by analyses of carboxyl-carbon radioactivities, *de novo* synthesis of palmitic and stearic acids by human epidermal preparations. With certain larger chain fatty acids (behenic, lignoceric and cerotic acids) there was evidence of successive elongation of unlabelled endogenous intermediates. Carboxyl-carbon radioactivities have not been studied here, however, because, with ^{14}C-valine, there was specific synthesis of isopalmitic acid and isostearic acid. This process requires the initiation of the fatty acid synthetase with a radioactive isobutyryl CoA moiety which is derived from valine, so that synthesis *de novo* of at least some of the observed radioactive lipids had occurred.

With all labelled precursors studied, radioactive monoenoic fatty acids were detected; and, with ^{14}C-acetate, traces of polyenoic C18 acids were seen. The positions of unsaturation in any of these radioactive products have not been established, however. There are much data describing the different series of unsaturated fatty acids in skin lipids (for example, Nicolaides and Ansari, 1968; Downing and Green 1968), however; and Wilkinson (1970b) has confirmed the presence of both a Δ 6 and a Δ 9 desaturase mechanism in human skin.

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Case Reports

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UNUSUAL FEATURES OF SCABIES ASSOCIATED WITH TOPICAL FLUORINATED STEROIDS

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Summary.—A baby with scabies had been treated topically with strong fluorinated steroid preparations for 4 months. He developed remarkably extensive scabies with many hundreds of burrows.

CASE REPORT

A healthy boy aged 3 months developed a rash which was treated by his general practitioner with betamethasone valerate 0·1% ointment and then with beclomethasone dipropionate 0·025% ointment.

The rash spread. After 4 months of treatment, when the baby was referred to hospital, he had raised red scabies burrows in profusion over the abdomen (Fig. 1), scalp, face, arms, legs, hands and feet. There were many hundreds of burrows, but few papules. Acari were found in scrapings of burrows from a foot and from the abdomen (Fig. 2). The baby was continually wriggling, movements which caused it to wander over the

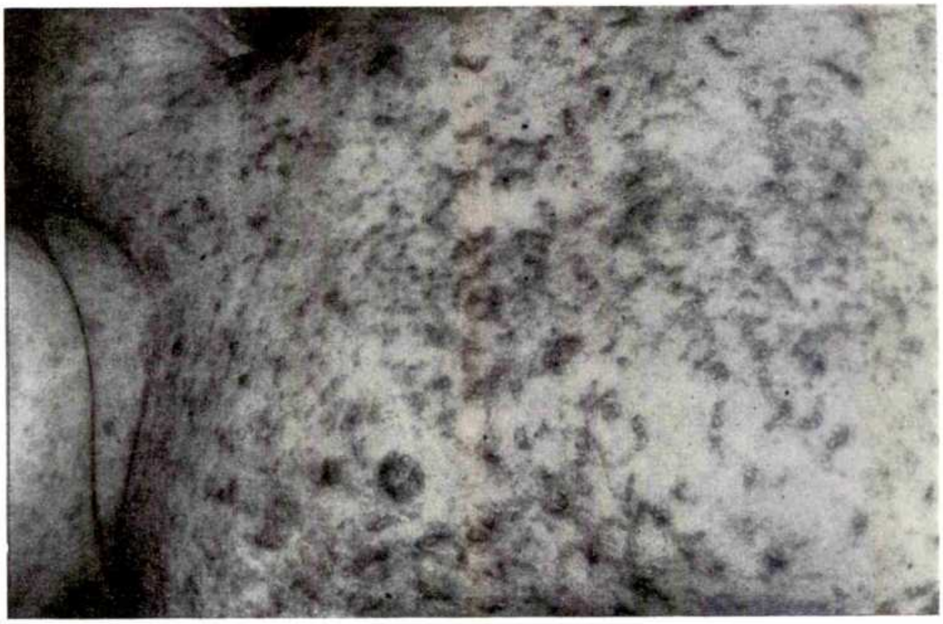

FIG. 1.—Abdominal skin with innumerable raised, red, zig-zag, linear burrows.

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examination couch, but he was not old enough to scratch himself. Blood contained 1,500 eosinophils/mm^3. The mother had mild early scabies. Treatment with Tetmosol (Monosulfiram 25%) diluted 1 in 4 was effective. When the baby was examined 10 days later, the sites of the burrows were marked only by lines of pigmentation in a pattern, which to the uninformed Western eye, recalled Chinese script.

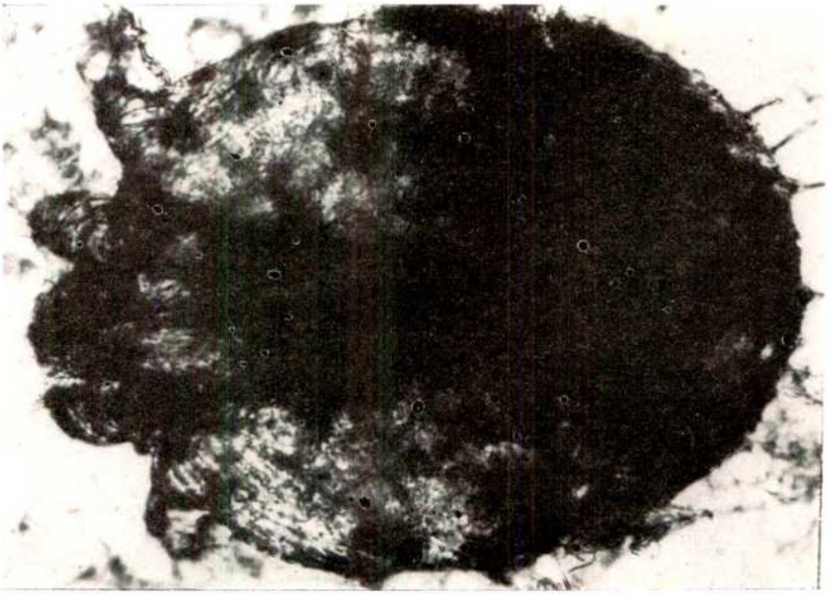

FIG. 2.—Acarus from burrow on abdomen (× 150).

DISCUSSION

In scabies the number of burrows usually does not exceed 10 (Heilesen, 1946), except in the crusted form occasionally found in institutionalized patients (Maguire and Kligman, 1960). This baby presented a clinical picture we have not seen before, and which is tentatively attributed to a hitherto undescribed, undesired effect of potent topical steroids.

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ERYTHRODERMA DUE TO PERITRATE AND GLYCERYL TRINITRATE

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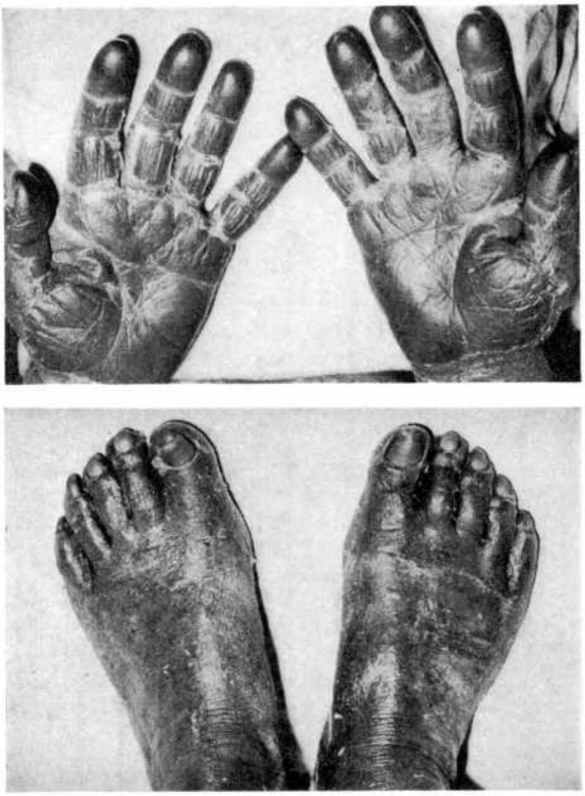
Summary.—Cutaneous sensitivity to peritrate (pentaerythritol tetranitrate) and glyceryl trinitrate is described, having developed simultaneously, despite the fact that the patient had previously taken drugs without mishap for 10 years.

PERITRATE and glyceryl trinitrate are vasodilator agents in general use for the treatment of angina pectoris. Cutaneous sensitivity to these drugs is known to occur occasionally and appears to be most commonly associated with peritrate (Goodman and Gilman, 1965). However, there do not appear to be any case reports of cutaneous sensitivity to these agents or of cross-sensitivity among the nitrate group of vasodilators in the literature.



FIGS. 1 and 2.—Oedema, erythema and desquamation of the hands and feet.

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CASE REPORT

Mr F., a retired steelworker aged 63, presented to the dermatology clinic in October 1971 with a 2 weeks' history of a rash. Of sudden onset, the rash started on the feet, extending up the legs to the trunk over 2 or 3 days. A few days later it also appeared on the hands, with a similar spread up the arms to the neck and face. The patient's description was that of a generalized red rash, with peeling of the hands and feet and with circular spots on the trunk. The hands and feet had also been swelling over the past week. There had been no recent illness or complaints, apart from an 8-year history of angina in the systematic enquiry, and there was no previous history of skin complaint in the patient or his family. His angina had followed a myocardial infarct 8 years previously. For this he had taken peritrate 10 mg four times daily continuously for 8 years, supplemented by glyceryl trinitrate taken when required (approx. 1 to 2 tablets monthly). No other drugs had been taken for at least 3 months prior to the onset of the rash.

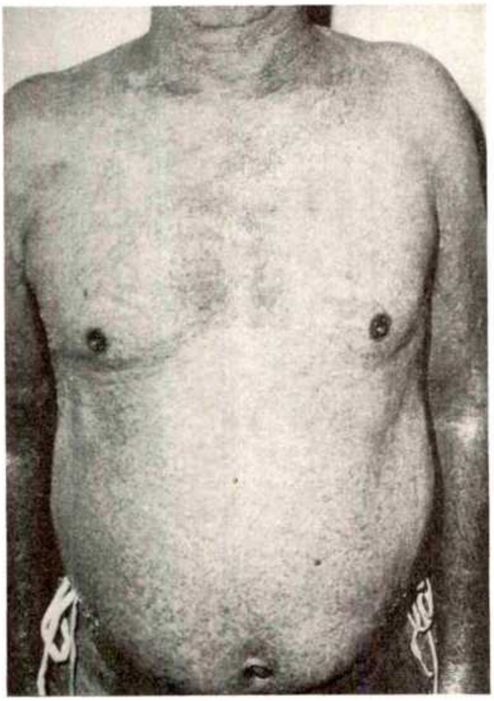

FIG. 3.—Early macular eruption on the trunk.

On examination he was found to have a bright red erythematous rash, with desquamation, confluent on the limbs, but still macular on the trunk (Fig. 1, 2, 3). The desquamation was particularly marked on the palms and soles and in the perineum. The affected skin was moderately itchy, but he complained of considerable soreness in the groins and perineum. There was also a marked pitting oedema of both legs as far as mid-calf, with a milder oedema of the hands and wrists. The only other positive finding was a firm, non-tender 3 cm hepatomegaly. Routine tests for fungal infection were negative.

On admission to hospital he was treated with local applications of 1% hydrocortisone cream and a bland lotion. Skin sensitivity to peritrate was suspected, and this drug was stopped. He suffered no angina at rest and therefore required no glyceryl trinitrate in hospital. Within 2 days the rash had improved and he was feeling better. Before resolution, the macular rash on his trunk became more obviously a confluent erythema; the feet and groins were more resistant to treatment, still showing some erythema and mild desquamation after 10 days. By discharge at 14 days he was practically clear.

However, he was given a supply of glyceryl trinitrate to take home. After taking only 2 tablets, and by the second day after discharge, the rash recurred. He was seen again at the clinic, when he appeared to have a milder form of the same rash as before. All tablets were stopped, and arrangements were made to re-admit him to hospital. During the three days' wait for admission his angina became intolerable, and he took in all 6 tablets of his own stock of peritrate. On admission, his rash had again worsened to a generalized erythroderma. A similar programme of bed rest and local therapy was instituted. As on the previous admission, one was impressed by the lack of systemic discomfort, his only complaint, apart from cutaneous symptoms, being a feeling of "coldness". He remained apyrexial. The W.B.C. count, E.S.R. and liver function tests were all normal. The rash settled again in 10 days to a mild scaly erythema of the groins and feet. Control of his angina was so important to him that sensitivity to these agents had to be tested objectively. Oral test doses of 5 mg (½ tablet) of peritrate and 1 tablet of glyceryl trinitrate each resulted in a recurrence of the rash, although it was noted that he reacted less to the glyceryl trinitrate than to the peritrate. The advice of a cardiologist was taken and, following a negative test dose, his angina was successfully controlled with oxprenolol (trasicor) tablets.

He has now remained well for 4 months, apart from a mild residual itching and desquamation of the feet.

DISCUSSION

If one views the formulae for the drugs mentioned above (Fig. 4), there is clearly a close structural similarity between glyceryl trinitrate and pentaerythritol tetranitrate, while oxprenolol is quite different. It would appear possible in this case that the trinitrate grouping has behaved as a hapten in exciting an immunological response.

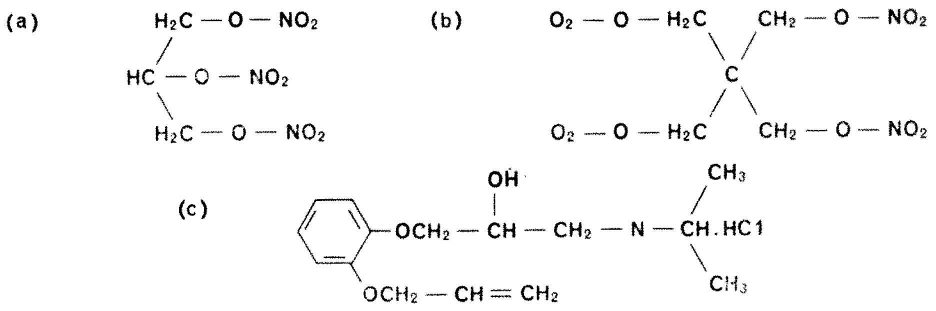

Fig. 4.—Structural formulae for (a) Glyceryl trinitrate; (b) Pentaerythritol tetranitrate; (c) Oxprenolol.

I should like to express my gratitude to Dr R. Church for his kind assistance in the preparation of this report.

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CROHN'S DISEASE OF THE MOUTH

L. STANKLER, S. W. B. EWEN AND N. W. KERR

Summary.—A patient with histologically proven Crohn's disease of the ileum and caecum presented with unusual ulceration of the mouth 3 years after subtotal colectomy. Worsening of bowel symptoms was associated with exacerbation of oral ulceration on several occasions. The areas of ulceration in the mouth were in relation to a sarcoid reaction typical of Crohn's disease.

A GRANULOMATOUS condition affecting the terminal ileum was described by Crohn *et al.* (1932), and the concept that this disease can involve other parts of the gastro-intestinal tract is widely appreciated. Crohn's disease may be restricted to the colon (Lockhart-Mummery and Morson, 1964) and appendix (Ewen *et al.*, 1971). Distal involvement of the colon (Comfort *et al.*, 1950) and anus (Gray *et al.*, 1965), and more proximal involvement, affecting the stomach and duodenum (Jones *et al.*, 1966), and oesophagus (Gelfand and Krone, 1968), are recognized complications of typical Crohn's disease. Recently, ulceration of the mouth has been reported in association with Crohn's disease (Dudeney, 1969; Issa, 1971; Bishop *et al.*, 1972; Croft and Wilkinson, 1972), and we now describe the fifth case with oral involvement.

CASE REPORT

A man aged 44 attended the Skin Department in May 1970 with a 6 months' history of recurrent oral ulceration. He also complained of persistent diarrhoea for 5 years, which necessitated a subtotal colectomy and ileocaecal anastomosis in February 1969 after a barium enema demonstrated changes in the terminal ileum, suggestive of Crohn's disease. At this time Mantoux tests at 1 in 10,000 and 1 in 1,000, and agglutination tests, were negative. Histological examination of the excised bowel showed the features of Crohn's disease (Fig. 1), and no acid-fast bacilli were identified. Family history was negative. The patient was found to have 3 oral ulcers and angular stomatitis. In-patient investigations revealed no evidence of malabsorption. On examination in the Dental Department in December 1970, he was found to have a most unusual linear oral ulcer in the vestibular sulcus, from which linear extensions enclosed areas of raised inflamed mucosa, giving rise to a granular or "cobblestone" appearance (Fig. 2), suggestive of Crohn's disease. A biopsy of the oral ulcer confirmed the clinical impression (Fig. 3, 4). Over the past year exacerbations of his bowel symptoms have frequently preceded or, on one occasion followed, outbreaks of oral ulcers.

DISCUSSION

According to Lennard-Johnes *et al.* (1968), the diagnosis of regional ileitis is dependent on a consideration of all the clinical and pathological features. There

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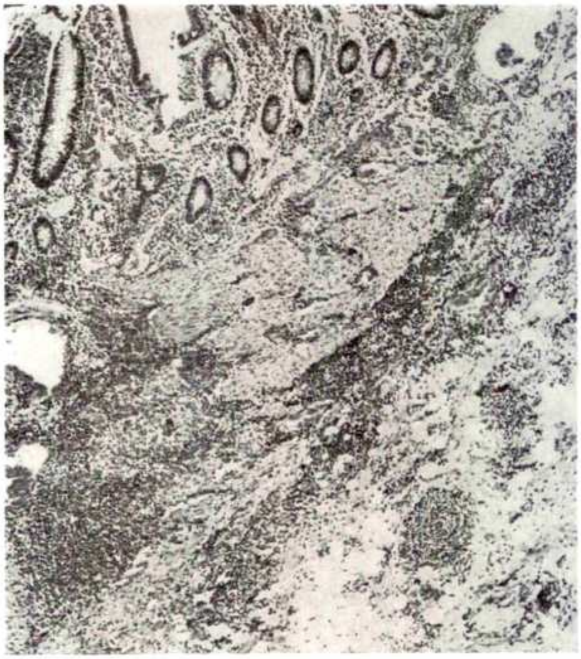

FIG. 1.—Caecum shows typical transmural inflammation, with a sarcoid reaction in the muscularis externa. H. and E. × 100.

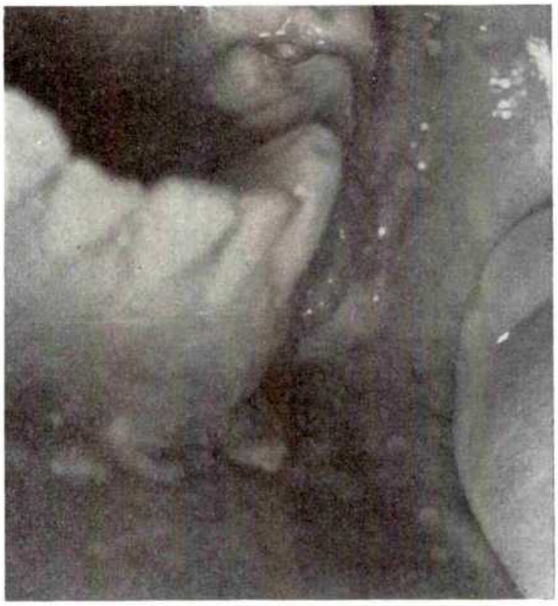

FIG. 2.—" Cobblestone " appearance of oral mucosa.

have been 4 previous reports of Crohn's disease of the mouth. Dudeney's (1969) case did not have a diagnostic histological oral picture (Stansfield, 1970), whilst the patient described by Issa (1971) had only suspected bowel symptoms, and the diagnosis was made on the characteristic oral pathology which cannot however be considered pathognomonic. The patient described by Bishop *et al.* (1972) had classical oral features, but histological evidence of intestinal disease was based on a rectal biopsy which showed possible granulomata only. In a recent review, Croft and Wilkinson (1972) found oral ulceration in 20 of 332 patients with Crohn's disease. In only one patient was histological examination of oral material carried out; this showed no evidence of follicle formation and was considered to represent a variant of Crohn's disease. The present patient had the clinical, radiological and pathological bowel findings of regional ileitis. The clinical appearance of his mouth, the coincidental exacerbations of his oral and bowel symptoms, and the presence of sarcoid granulomata seen on examination of oral biopsy material supports the belief that Crohn's disease of the mouth is a clinical

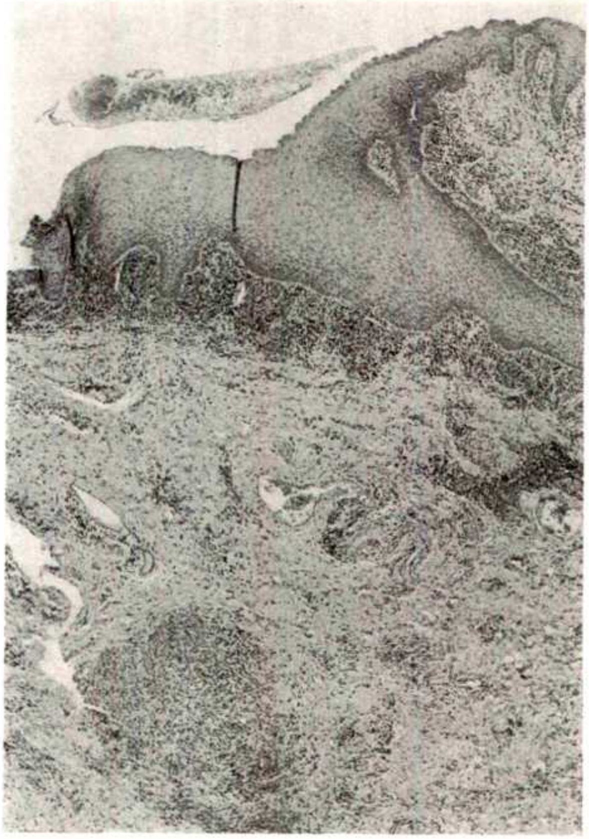

FIG. 3.—Oral mucosa adjacent to an area of ulceration shows a sarcoid reaction in the underlying connective tissue. H. and E. × 100.

entity. The classical oral appearance had been missed in 2 reported cases, and it seems quite possible that mouth involvement is commoner than is generally supposed. Linear oral ulceration with a " cobblestone " appearance should lead one to suspect the diagnosis, which is strengthened by finding a characteristic histological picture.

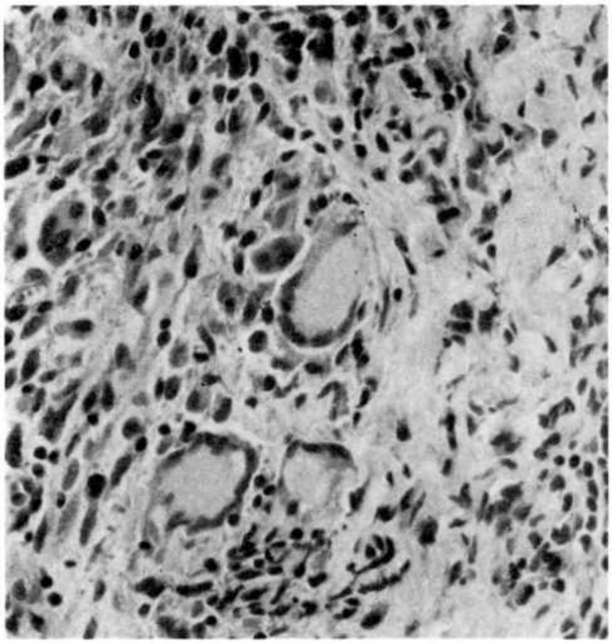

FIG. 4.—High power of sarcoid reaction in Fig. 3 shows aggregate of epithelioid cells, giant cells and lymphocytes. H. and E. × 600.

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A CASE OF TINEA NIGRA IN BRITAIN

H. W. CHADFIELD AND C. K. CAMPBELL

Summary.—A case of tinea nigra occurring in a Caucasoid girl aged 10 is described. The infection was probably contracted in Malaya where she had lived between the ages of 2 and 4½. The course of the infection was chronic and protracted, with a tendency to recur after apparent cure. The mycological features are described and the aetiology is discussed.

TINEA NIGRA, caused by tropical species of the dematiaceous fungus: *Cladosporium*, is rarely reported in Britain. The most recent case (Fountain, 1968) probably originated in the West Indies, and before that Sharvill (1964) described a combined infection with *Microsporum canis,* believed to have been contracted in S. Africa.

The present account describes a further British example, probably originating in Malaya.

CASE REPORT

The patient was a girl who was born in Britain in 1962 and who had lived in Malaya between the ages of 2 and 4½ years. Her mother stated that a small (1 cm²) pigmented area at the base of the web between thumb and index finger had certainly been present when the child began school in England at the age of 5, but she was not sure of the exact date of onset. Its first appearance may have been several years earlier. The lesion had spread slowly since that time, with darker areas occasionally appearing. There was an acceleration of its growth rate about 3 years prior to the first referral to the clinic.

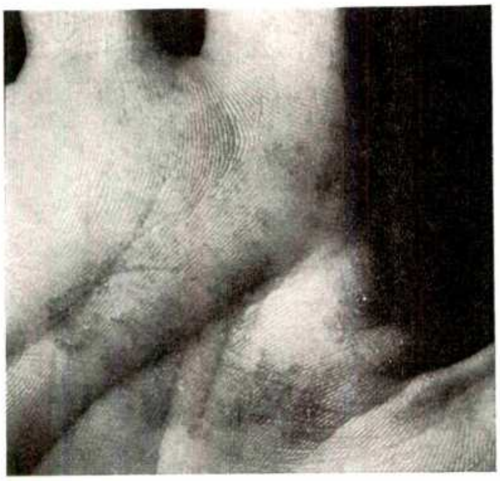

FIG. 1.—Initial appearance of lesion.

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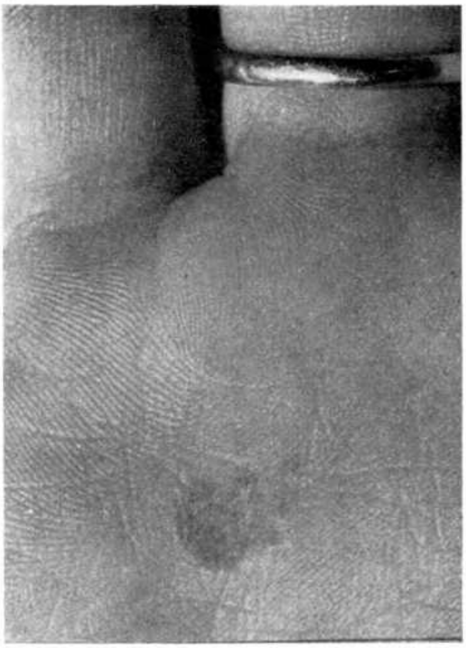

FIG. 2.—Relapse of lesion after apparent cure.

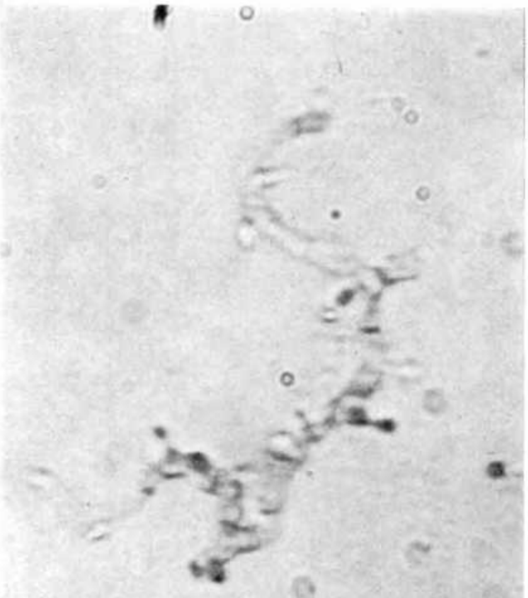

FIG. 3.—Microscopic appearance in KOH mount of lesion.

There were then no pets in the family, apart from a canary acquired in January 1971.

When she was first seen at the clinic at the Corbett Hospital, Stourbridge, on January 19th, 1971 there was a flat, brownish figurate, marginated and mottled, slightly scaly area, with fainter satellite lesions on the right palm (Fig. 1). The first impression was of a pigmented naevus of junctional type—numerous such lesions were present on the neck, trunk and outer margin of the right foot—but tinea nigra was considered a possible diagnosis, and scrapings of the lesion were obtained and examined mycologically (see below).

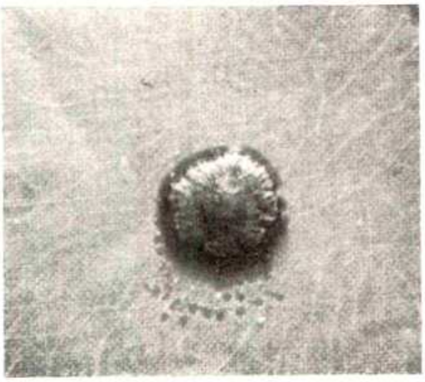

FIG. 4.—Culture of lesion.

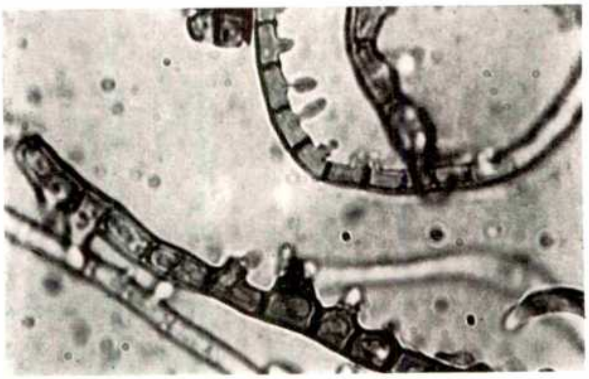

FIG. 5.—Microscopy of culture.

Treatment and progress

Whitfield's ointment was prescribed at the time of the patient's first attendance and, when she was seen a month later, the lesion had completely cleared clinically and treatment was discontinued. She was asked to return in 6 weeks for review, and there was then no clinical evidence of recurrence. Seven months later, however, in October 1971, she was again referred by her general practitioner because the lesion had recurred. It now consisted of a small, pigmented, finely scaly macule, with a crenated margin and tiny satellites situated distally on the palm (Fig. 2). Treatment with Whitfield's ointment was re-instituted and, when seen on December 14, 1971, there was no evidence of the lesion. In view of its past behaviour, however, treatment was ordered for a further 2 weeks. When seen again on January 28, 1972, however, further lesions had appeared over and contiguous to the previously affected sites. On February 2, 1972 these had become more prominent. This experience is contrary to the statement of Beare *et al.* (1968) that the condition yields to the topical application of Whitfield's ointment, but there is evidence that treatment may not have been carried out very consistently and this could explain the marked tendency to recur.

Mycology

Scrapings of the original lesion were received in the laboratory 17 days after they were taken, the delay being due to a postal strike. Skin mounted in 15% KOH was found to contain short lengths of dark, olive green, septate hyphae (Fig. 3). Some hyphae bore short, lateral, bud-like projections. Skin scales were inoculated on to malt extract agar incorporating 0·05 mg/ml chloramphenicol, but incubation at 27°C for 2 weeks failed to grow the organism.

The second sample from the palm, received on November 11th, 1971, contained a fungus with the same appearance as the first. On this occasion culture was successful, the fungus appearing after incubation for a few days at 27°C on malt agar as a raised, olive black, velvety colony. After 2 weeks the growth had reached a diameter of 1 cm and the surface was smooth and yeast-like (Fig. 4). Microscopical examination revealed the production of blastospores as lateral buds from curved, unilaterally-thickened conidiophores (Fig. 5).

DISCUSSION

Conant *et al.* (1971) and many other authorities now attribute all case of tinea nigra to *Cladosporium werneckii*, first isolated and described by Horta in 1921 from Brazil. Reports of its occurrence have come from several American countries and South Africa. Similar pigmented lesions discovered earlier in the Far East by Castellani (Castellani, 1964) have been attributed by Conant *et al.* to pityriasis versicolor. However, as Luries and Borok (1958) have pointed out, the fact that Castellani isolated *Cladosporium mansonii*, a closely similar species to *C. werneckii*, from these sites indicates that a form of tinea nigra may occur in an area embracing Ceylon, India, Burma and Malaysia. The question of whether *C. werneckii* and *C. mansonii* are identical is not yet resolved, but at least on serological criteria there is reason to believe they are distinguishable (Dr Helen Buckley, personal communication).

The cultural characteristics of the fungus isolated from the present case are consistent with those of the above species, but a serological study would probably be necessary to carry the identification further. The clinical features of this case are in conformity with those previously described, although an absence of scaling is noted by most authors.

In view of the infrequency with which this condition is reported in Britain and of the association of this patient with Malaya, it seems likely that the infection was contracted in the Far East and had persisted undiagnosed for some 6 years.

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Comment

HAIR REPLACEMENT IN THE GUINEA-PIG AND IN MAN

Except in the Merino sheep, in which continuous activity is maintained throughout life, formation of hair by individual follicles is intermittent in all species so far studied. In most mammals, hair replacement is more or less synchronous in each region, but not, in the adult, over the whole body; the process is drawn out so that any severe or sudden change in thermal insulation is avoided. Thus in the mouse and in the rat, the cycles in the ventral follicles usually run in advance of those in the dorsal so that there are successive waves of hair replacement which start on the belly and move over the flanks to the back.

Moulting is influenced by environmental factors, of which the chief is the photoperiod, acting through the hypothalamo-hypophyseal system, possibly with mediation by the pineal (Ebling and Hale, 1970). At the same time, hair follicles appear to have intrinsic rhythms which are slowly influenced by hormonal or other systemic factors (Ebling and Johnson, 1964a). Gonadal, adrenal and thyroid hormones may all play a part. Hooker and Pfeiffer (1943) and Light and Tornaben (1953a, b) were among early workers who showed that oestrogens inhibited hair growth. This was later confirmed by Johnson (1958b), who went on to show that spaying of female rats increased hair growth.

In the scalp of man each follicle has been described as behaving independently of its neighbours so that there is a " mosaic " pattern of hair replacement (Chase, 1954; Chase and Eaton, 1959). The guinea-pig (Dawson, 1930; Trotter and Dawson, 1931; Strangeways, 1933; Bosse, 1965) is also reputed to show a mosaic moult, and has often been regarded as a model relevant to human studies. If moulting in these two instances is a continuous process, one might expect that the hair follicles will not retain their sensitivity to steroid hormones (Jackson and Ebling, 1972). However, in the human scalp, it appears that post-partum hair loss may be an effect of hormones circulating during pregnancy and their subsequent withdrawal. Moreover, in the guinea-pig, topically applied oestradiol has been shown to prevent regrowth of hair on denuded patches (Whitaker, 1956). It has seemed important therefore to try and analyse further the effects of oestrogen on hair follicles which reputedly show a mosaic pattern of activity.

Recent studies on hair growth in normal female short haired guinea-pigs (Jackson and Ebling, 1971) and in spayed animals (Jackson and Ebling, 1970, 1972) has provided new information on the mosaic pattern. It has become clear that in normal guinea-pigs, as in the rat (Johnson, 1958a), the definitive length of hair varies from region to region. Such differences are brought about by variations in both the rate and duration of growth. Further, coarse hairs have higher rates of growth than thinner ones. Treatment with oestradiol clearly and significantly reduces the rate of growth, whether in comparison with spayed or intact animals. Thus the hair follicles of the guinea-pig, with a presumed mosaic pattern of activity, respond to oestrogen in the same way as those of the rat, in which activity occurs in a patterned sequence. The demonstration confirms Danforth's (1925) supposition that the ovary inhibits hair growth, and explains Dawson's (1933) finding that regrowth of hair in clipped mature guinea-pigs was higher in spayed females than in intact virgin or breeding females. In spite of the clear effects of oestradiol on the rate of hair growth, it appears to have no effect on the duration of anagen. Changes in the duration of anagen have never in fact been produced in any experimental animal. At the same time, it is difficult to believe that the abnormally high proportion of active follicles in the human scalp in late pregnancy is not a result of the prolongation of anagen, due to circulating hormones, probably oestrogens. Oestradiol also alters the duration of the resting period, i.e. by delaying the initiation of anagen. Johnson (1958b) demonstrated clearly that initiation of anagen in ventral follicles of the rat could be delayed by more than 5 weeks. In the guinea-pig, oestradiol not only delays the initiation of anagen in some follicles, but also appears to prevent it in others. Thus the guinea-pig, in spite of its reputed lack of pattern in the moult resembles the rat in its response, oestradiol acting, as in the rat, to reduce the rate of hair growth and to delay the initiation of follicular activity.

The new evidence also raises the question of whether the guinea-pig moult can be properly described as a mosaic. It has become clear that whilst there is no synchrony between the follicles of different fibre types, there is a marked degree of synchrony *within* each fibre type. This synchrony is clearly recognizable over the first 48 days of life and there is evidence to suggest that a measure persists until 150 days and possibly beyond. These facts suggest that the guinea-pig, in common with the rat, mouse and other rodents, may possibly have a wave pattern of activity, albeit obscured by the lack of synchrony between follicular types. This view has received support from the work of Tejima *et al.* (1968) who found a peak in the anagen/telogen ratio which moved in time from head to tail. This led them to suggest that waves of hair growth were moving from anterior to posterior.

How far does hair growth in man resemble that in the guinea-pig? The supposition that the pattern of follicular activity in man is a mosaic is brought into question by several pieces of evidence. Firstly, hair follicles occur throughout the body in threes and within each trio group the hair cycles appear to be synchronized, though their periods vary with region, ranging from about 3 months in the moustache to 8 months in the leg (Saitoh *et al.*, 1970). Secondly, the scalp of the newborn appears to exhibit waves of hair growth. Pecoraro *et al.* (1964) found at this stage a large measure of synchrony in any given region, with anagen generally predominating, but also with a much higher proportion of follicles in catagen than in the adult. They concluded that at birth a wave of hair growth was passing over the scalp from the frontal to the occipital region. Thirdly, Orentreich (1969) has found that in the North Temperate Zone short-term hair growth cycles are evident and are superimposed on a longer seasonal cycle with a maximum defluvium in November. A seasonal difference of 30% exists between the minimum and maximum hours of daylight in this zone, whilst the average mean temperature ranges from 14·4°C in winter to 28·3°C in summer. Either or both of these seasonal variations may be the cause of the maximum defluvium in November, for at the equator, where the photoperiod is constant, there is no annual effect on the hair growth cycle. Finally, a study of the diameter of scalp hair from women with diffuse hair thinning has suggested that in the development of this condition not all follicles behave identically, and that there may be more than one *type* of follicle in the human scalp (Jackson *et al.*, 1972). These considerations lead me to suggest that there may be no true examples of a random "mosaic" type of follicular activity as distinct from a "wave" pattern. Wave patterns may become obscured not only because different follicular types are out of phase with each other, but because successive waves come to overlap. Such overlapping has been described in the rat (Ebling and Johnson, 1964a, b) in which the duration of anagen is 12–19 days; it is even more likely to occur in the guinea-pig with a period of 30–40 days, or in the human scalp where follicles can remain active for 2 years or more (Kligman, 1959, 1961).

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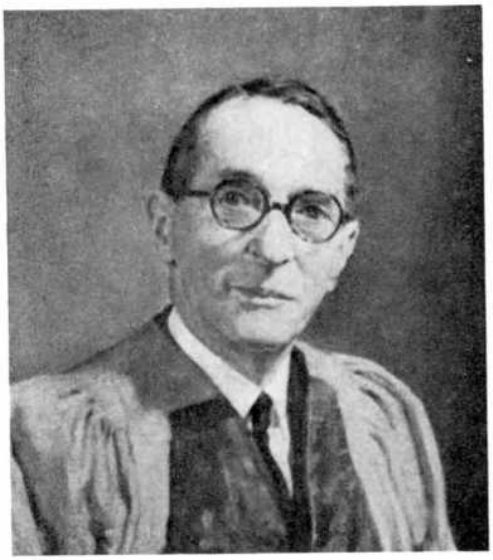
Obituary

JOHN THORNTON INGRAM 1899–1972

John Ingram was convinced that dermatology was one of the most important, if not the most important, subject in medicine and as such warranted an equal status with any other branch. This conviction he pursued with such energy that he gradually impressed his general medical colleagues so that they developed an increased respect for the study of skin diseases at a time when dermatology was considered by most physicians as only a second rate subject. This influence was shown following his appointment in 1927 as physician in charge of the newly formed skin department in Leeds at the age of 28. Within 12 years he had persuaded the authorities to build a skin department in the new out-patient block which for years remained second to none in the country. Dermatology also took a prominent place in the curriculum and was fully represented in the final examination with the only external examiner in the country; this greatly enhanced the importance of the subject in the eyes of the students who had already spent an intensive three months at " skins " during their training. Several times during the war the writer was told that an ex-Leeds student could be recognized because he knew his " skins ". In this department he developed a large lupus clinic in which no patient was pronounced cured till he had been clear for five years, and also the intensive treatment of psoriasis, largely on an out-patient basis, which has become known as the " Ingram treatment ".



John Ingram was a very careful observer and the histories of his patients and the descriptions of their lesions were meticulously recorded. He always insisted on a thorough general examination of the patient at a time when most dermatologists were only paying lip service to this. Trained at the London Hospital under Sequeira and with O'Donovan, he was very conscious of the importance of psychological factors in skin diseases and he was prepared to spend long sessions with individual patients even in out-patients. This approach was further expanded by his close co-operation with his lady almoner in dealing with both psychological and social problems.

In 1939 he joined the army and went to France with the B.E.F. holding the rank of

Colonel. Here he had differences of opinion with the authorities as he believed strongly that army dermatology should be separate from venereology with its own independent adviser at the War Office. He also urged that skin wards should be under the control of nursing sisters and not men as in the V.D. sections. He returned to civilian life after Dunkirk but his ideas were later implemented in the army.

He remained in Leeds till 1959 making it an outstanding dermatological centre and attracting many overseas graduates especially from Australia, and building up a school of young dermatologists bearing the imprint of his exacting discipline. On the retirement of Sydney Thompson at Newcastle, he was asked to advise on the re-organization of the skin department there with a view to creating the first whole time chair in the U.K. However, when this was done, no suitable candidate could be found and so he himself was asked to fill the post. He was thus able to use his experience and great influence to lay the foundations of another outstanding department which, when he retired in 1963, he could hand over to his successor in full running order. Tragically his retirement was marred by ill health and he was never able fully to become the active elder statesman which should have been his destiny.

Ingram wrote on many subjects, especially psoriasis but possibly his most outstanding contribution was the part authorship of Sequeira's " Diseases of the Skin ", much of which was a very personal expression of his own experience. He was very active in other fields holding many offices in his own hospitals, serving on the G.M.C. and on the Council of the R.C.P. and on several M.R.C. Committees. He was President of the B.A.D. in 1947 and of the Dermatological section of the R.S.M. in 1957. Space prevents a longer list but this may be found in the B.M.J. for July 1st, on page 54.

John Ingram was rather a shy man but in his own home he was a charming and often amusing host and many overseas visitors recall his hospitality with gratitude. He married Lucy Graham, who like John was small in stature but the two of them made a very positive and popular couple at meetings, at home and overseas. Lucy by whom he had his devoted daughter Pamela, died in 1957 and two years later he was remarried to Kathleen Raven, formerly Matron of the General Infirmary, Leeds and later Chief Nursing Officer for the Department of Health and Social Security.

F. F. Hellier

Br. J. Derm. (1972) **87**, 514.

Society Proceedings

IRISH DERMATOLOGICAL SOCIETY

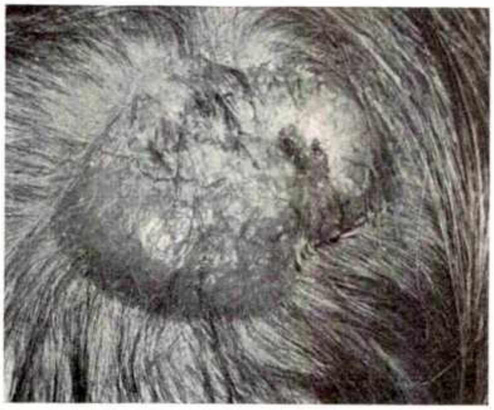
Dublin, April 29th, 1972

Chairman—Dr D. Mitchell

Mycosis Fungoides Presenting as a Solitary Tumour.—Dr D. Mitchell.

A man aged 68.

History.—Two years ago he noticed a pimple on the top of his head which has gradually enlarged to its present size. No pruritus.



On examination.—On the vertex there is a hemispherical nodule 5 cm in diameter with a thick crust on top. When this is removed the epidermis appears intact with obvious dilated venules (Fig. 1). No other abnormality in skin or on general examination.

Histology (17.1.72).—The lesion is composed of abnormal histiocytes interspersed with occasional neutrophils and eosinophils, together with plasma cells and lymphocytes. Mitotic figures are numerous and abnormal mitoses are seen. "Mycosis" cells are present. All of these cells occupy the dermis and underlying tissue. Mycosis fungoides in the tumour stage.

Blood count normal.

Treatment.—5% 5-Fluorouracil cream was applied twice daily without occlusion for several weeks with apparent flattening of the tumour mass which, at the same time, increased in area. Later the tumour was irradiated with 600 r at 100 kV with no immediate response.

Comment.—In the cases of mycosis fungoides described by Block *et al.* (1963) 5 out of 33 developed tumours without preceding eruptions; the histology in all cases was characteristic of mycosis fungoides.

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Br. J. Derm. (1972) **87**, 515.

Books

Protection Against Radiation from Brachytherapy Sources. Recommendations of The National Council on Radiation Protection and Measurements, N.C.R.P. Report No. 40. (1972) Washington: NCRP Publications. Pp. 65+ vi. Price: £1.00.

The National Council on Radiation Protection and Measurements is a corporation set up by Congress to make recommendations with regard to radiation. The work under review is their standard manual about sealed sources for short distance medical work. It brings together technical expertise both from the clinical and scientific points of view under the chairmanship of E. L. Safnger and supersedes Report No. 24 of 1960. It will be immediately recognized as authoritative and sound and although it is intended only as a code of practice and does not have the force of regulations there can be little doubt that in the U.S.A. these are the rules which everyone will go by. It will be mandatory reading for all medical radiation workers and hospital administrators in the U.S.A. and also for gynaecologists and dermatologists owning radium, beta ray plaques or other sources. It will be of rather less value in the U.K. which has its own Code of Practice for use in National Health Service Hospitals; there are also regulations set up by the Ministry of Transport. These are very much more detailed and strict and they do have to be observed.

The British dermatologist is much less likely to possess his own sources of radiation than his counterpart in the U.S.A. but the manual would be of interest to Australian dermatologists or those in developing countries. The Report describes measures to be taken in minimizing unwanted radiation to the public generally and to patients and radiation workers; the design of containers for storage and transport; sterilization and cleaning of sources and systems of documentation to prevent accidental loss and contamination. It also describes methods of supervision and personal monitoring. It includes a short glossary of technical terms and tables giving physical data on the commoner radio-active sources and shielding materials.

P. T. CHOPPING

Periodicals

Arbeitsmedizin, Sozialmedizin, Arbeitshygiene (1972) **7**, *149–168.*

The greater part of the June issue of this journal is devoted to a series of papers on occupational mycotic infections. These papers are valuable because they combine their authors' personal experience with an extensive review of the widely scattered international literature. Sonck writes on Medical and Veterinary personnel, Forck on the Food Industries, Brehm on Agriculture, Fabry on Mining, Rieth on Immigrant Workers, and Weber and Dausch on practical laboratory problems.

American Zoologist (1972) **12**, *13–171.*

In December 1971 a Symposium on The Vertebrate Integument was arranged by Dr P. E. A. Maderson for the Division of Morphology of the American Society of Zoologists. The 12 papers now published in the American Zoologist present admirably up to date and well documented summaries of the present state of knowledge of many aspects of the comparative morphology and physiology of the vertebrate integument. Copies of the Symposium may be purchased for $2.00 from Mrs Mary Wiley, Business Manager, American Society of Zoologists, Californian Lutheran College, Box 2739, Thousand Oaks, California 91360, U.S.A.

Br. J Derm. (1972) 87, 516.

Correspondence

Separation of Stratum Corneum

SIR.—Concerning the interesting article by Dawber *et al.* (1972), we should like to add some of our own observations on the use of ethyl cyano-acrylates (permabond) in skin biopsy. Our study is concerned mainly with the bacterial flora of the skin and their changing pattern in the presence of antimicrobial agents incorporated in various topical preparations. During the course of our work we found that, for mounting purposes, the biopsies taken from the volar region of the arm could be separated from the glass slide by immersing these slides in a solution of 1–2% Tween-80 (polyoxyethylene sorbitan mono-oleate) for 30–35 min rather than by the use of a razor blade. A quicker separation, if needed, could be obtained by the use of a 0·025 mol/l NaOH solution for 5 minutes. Tween-80 is preferable because of its non-activity with skin proteins (Lansdown and Grasso, 1972).

By using a modified method of Hucker's Gram stain (modifications: decolourization with 80 : 20 alcohol/acetone solution for 30 seconds and counterstaining with safranin for 2–3 min), we were also able to detect the presence of bacteria in these permabond skin biopsies.

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Action of Staphylococcal Epidermolytic Toxin on Mouse Skin in Organ Culture

SIR.—Dr H. M. McCallum's finding (1972) that staphylococcal epidermolytic toxin in organ culture of skin from newborn mice produces epidermal separation between the stratum granulosum and spinosum, prompts us to report similar findings in organ culture of human skin.

We studied the effect of a keratolytic protease derived from a species of *Cytophaga* on human skin maintained in an organ culture system similar to that employed previously (Sarkany and Gaylarde, 1970). After periods in culture for up to 3 days, the skin was fixed in formol-saline and histological sections were made. At the end of 3 days, almost complete disaggregation of the epidermal cells was noted as a result of exposure of the skin to the enzyme. Earlier changes were separation of the basal layer from the stratum spinosum and separation of the horny layer from the rest of the epidermis (Fig. 1).

In culture media containing a high proportion of serum, there are morphological alterations characterized by lack of stratum granulosum formation and abnormal keratinization resembling parakeratosis. In synthetic tissue culture media, such as TC-199, normal morphology is retained longer, but this is accompanied by depression of biochemical activity, virtual cessation of DNA synthesis and inhibition of cell migration (epiboly) around the free borders of the dermis. Our results show that a keratolytic protease is capable of producing a condition similar to the changes seen in toxic epidermal necrolysis. This fits in with the changes described with other proteolytic enzymes, characterized by cell disaggregation, cleft and blister formation.

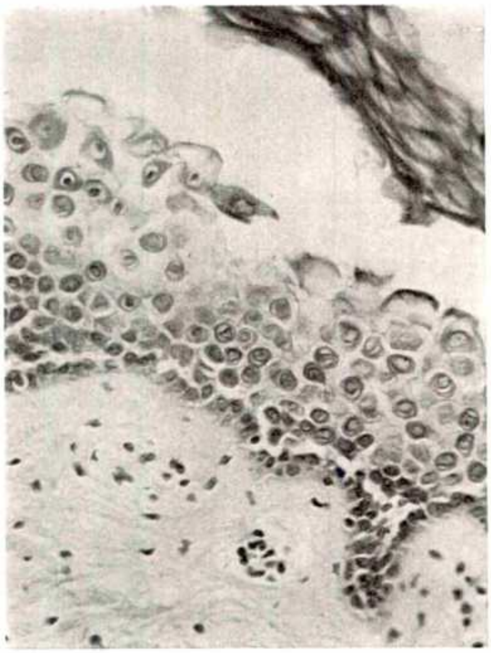

FIG. 1.—Human skin cultured in the presence of keratolytic enzyme showing cleft formation, loss of cytoplasmic staining and changes in nuclear morphology. ×150.

Mouse epidermis is only 2–3 cell layers thick and makes precise localization of changes produced by enzymes difficult. The alterations in human skin are also more relevant to the study of the problem of toxic epidermal necrolysis. We would, therefore, suggest that the human organ culture system would be more suitable for investigations of the effects of staphylococcal epidermolytic toxin. It is likely that this substance is in fact proteolytic enzyme.

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We thank Glaxo Laboratories for providing the keratolytic enzyme used in this study.

Br. J. Derm. (1972) 87, 518.

Quarterly Review

THERAPEUTICS XX

The Management of Deep Venous Thrombosis

Part I—Diagnosis

It has been known for some time that the incidence of deep thrombosis found at post-mortem is greatly in excess of that diagnosed clinically (Rössle, 1937; Neumann, 1938; Frykholm, 1940; Hunter *et al.*, 1941). New diagnostic procedures have enabled us to demonstrate this higher incidence of thrombosis during life.

Deep venous thrombosis may lead to pulmonary embolism resulting sometimes in death or in chronic pulmonary hypertension. Locally, the thrombosis may destroy the effect of the muscle-venous pump in the legs. Efficient venous flow from the legs depends upon the competence of the valves in all three venous systems, deep, perforating and superficial. Following thrombosis, valves are either destroyed during recanalization or rendered incompetent due to dilatation of collateral vessels. Destruction of valves in the lower perforating vessels leads to superficial venous hypertension, gravitational skin changes and varicosities. Thrombotic occlusion of major veins in the leg, even if followed by recanalization or adequate collateral circulation, frequently leads to chronic dependent oedema.

It is the newer diagnostic procedures and the implications of their use which form the subject of this review.

Phlebography.—This is the most useful method of diagnosis, is fairly simple and demonstrates between 90–95% of venous thrombi (Browse *et al.*, 1967, 1969). Whilst the superficial veins are firmly occluded at the ankle a radio-opaque dye is injected into a vein on the dorsum of the foot. Compression causes the dye to shunt into the deep calf veins and by dorsiflexion of the foot it may be pumped into the popliteal, superficial femoral and common femoral veins. The iliac veins and inferior vena cava may not be demonstrated by this injection alone, therefore a second injection of contrast medium into the femoral veins may be needed. If no vein is available for these injections the dye may be introduced intraosseously either at the ankle or into the upper end of the femur. These injections give good venous filling (Thomas and Fletcher, 1967) but are much more painful for the patient so that a general anaesthetic is often required.

Interpretation of phlebograms is not difficult (De Weese and Rogoff, 1963; Haeger and Nylander, 1967). "Streaming" of contrast medium, the appearance of valves and variable mixing are the most common source of diagnostic error. Some veins such as the profunda femoris usually fill badly. As the thrombus gets older, retracts and becomes adherent, the appearance is more difficult to interpret particularly if the thrombus is viewed *en face*, when thinning of the dye stream may be almost imperceptible. Fresh non-adherent thrombus, however, is outlined on all sides by dye and when seen on screening appears as a frond waving in the blood stream. Such screening also provides other useful information concerning blood flow characteristics and reduces the number of false positive reports.

I^{125} labelled fibrinogen.—This technique was first described by Hobbs and Davies in 1960. Its use has been firmly established (Atkins and Hawkins, 1965; Nanson *et al.*, 1965; Flanc *et al.*, 1968; Negus *et al.*, 1968). Human fibrinogen labelled with 100 μCi I^{125} is injected intravenously, I^{125} being used because of its long half life of 60 days.

Potassium iodide in a dose of 150 mg is given orally 24 hours prior to the injection and continued daily for 3 weeks. This prevents the uptake of I^{125} by the thyroid and possible damage by irradiation. Since the labelled fibrinogen is completely dispersed in the intravascular compartment, counts over the heart represent a baseline level. Counting is done with a collimated instrument at measured intervals from the bifurcation of the inferior vena cava down the medial aspect of each leg. Measurements are performed daily, thus changes may be related to time, the other leg and the baseline count over the heart. Since the fibrinogen is converted to fibrin, fixed and concentrated during thrombosis, a persistent local elevation of counts of 15–20% above the baseline is diagnostic of active thrombosis (Negus *et al.*, 1968).

This technique has the advantage of simplicity and causes little discomfort to the patient. It can be used at the bedside.

The major disadvantages are the length of time taken by potassium iodide to protect the thyroid and the delay before the labelled fibrinogen is incorporated into the thrombus. In practice this means that there is at least a 24-hour delay before a diagnosis can be made. If thrombosis has been present for more than 5 days this also makes interpretation difficult unless there is definite propagation of thrombus because the older thrombosis takes up fibrinogen less actively.

However, within these time limits, results of 80–90% accuracy have been obtained for thrombosis in the thigh and calf veins (Browse *et al.*, 1971). In the iliac and upper femoral veins this accuracy is considerably reduced.

Other factors which reduce the reliability of the technique are healing wounds and oedema of the legs since in both circumstances a local increase in fibrinogen concentration occurs, the former by incorporation and the latter by leakage from capillaries.

The use of I^{125} is contraindicated in pregnancy and should be carefully considered in young people. Although the risk of serum hepatitis must now be very small this has to be borne in mind.

Ultrasound.—This is also a technique causing little disturbance to the patient. It depends upon the Doppler principle in which the frequency of a reflected ultrasonic beam is altered by particles in the blood in a way which is directly proportional to their velocity. Thus, any obstruction in the venous system reducing this flow rate can be demonstrated. The accuracy of the test is further increased by augmenting the blood flow. This is achieved either by squeezing the thigh or calf, or by sharp dorsiflexion of the foot (Evans and Cockett, 1969; Evans, 1971). There is a possible risk however of detaching a thrombus during this manoevre (Browse, 1969). Many ultrasound detectors are available commercially, one frequently used is manufactured by Sonicaid Ltd (Evans and Cockett, 1969).

Ultrasound will only detect thrombus in large vessels above the popliteal vein and it is of limited use once collateral circulation has been established and also in partial venous occlusions. It does not reveal the position and state of the thrombus. Speed and simplicity are the advantages of the ultrasound method. The accuracy of this method as compared with phlebography is about 90% (Evans and Cockett, 1969).

Haematological Investigations

Certain of these tests may be of value in detecting a thrombus and can indicate the underlying cause.

Studies of platelet adhesiveness have shown the possible value of this measurement in predicting deep venous thrombosis (Bygdeman *et al.*, 1966; Hume, 1966), though its usefulness has been questioned by others (Negus *et al.*, 1968).

A good guide to the blood coagulability can be obtained by separation of coagulation products in the serum using gel filtration (Fletcher *et al.*, 1970). This may be a useful diagnostic aid in the future.

Certain diseases including monoclonal gammopathies, cryoglobulinaemia, macroglobulinaemia and polycythaemia predispose to thrombosis and these can often be detected by routine haematological investigations.

A generalized intravascular coagulation process in its early stages may present only as a deep vein thrombosis. The finding of burr cells, uraemia, fibrin degradation products and hypofibrinogenaemia may suggest a more generalized disturbance.

Other Tests

Changes in electrical impedance occur in all body compartments with alterations in their fluid volume. In the leg, such changes occur in time with the respiratory cycle because of fluctuations in venous pressure. These changes will be reduced by the presence of a thrombus in a major vein, and can be detected using suitable instruments (Mullick *et al.*, 1970).

A further method is to use infra-red photoscanning to detect " hot spots " produced by thrombophlebitis (Lane, 1967).

Neither of these techniques has been fully evaluated but it seems unlikely that their usefulness will be greater than existing methods.

Discussion

The application of these diagnostic aids has confirmed 3 facts. Firstly, that the incidence of venous thrombosis in all patients is much higher than formerly believed, ranging from 28–35% in different series (Flanc *et al.*, 1968; Negus *et al.*, 1968; Browse and Negus, 1970). Secondly, thrombosis may not be present when clinical signs suggest its presence (Sevitt and Gallagher, 1961). Finally, the onset of thrombosis most frequently occurs at the time of operation or within the first 5 days of confinement to bed (Browse and Negus, 1970). Furthermore, several groups of patients particularly at risk of developing thrombosis have been identified. These include the elderly, particularly if immobilized (Sevitt and Gallagher, 1961; Sharnoff and Rosenburg, 1964), patients undergoing hip surgery, splenectomy, pelvic surgery and prostatectomy. Myocardial infarction is also associated with a higher risk of thrombosis (Murray *et al.*, 1970; Nicolaides *et al.*, 1971). The obese or very tall patient also seems to be more at risk than the patient of average build (Little *et al.*, 1966; Richards, 1966; Vessey and Doll, 1969; Naide, 1952). Oral contraceptives and pregnancy predispose to venous thrombosis (Vessey and Doll, 1969; Daniels *et al.*, 1967) although this has been disputed (Drill and Calhoun, 1972).

Patients in these " high risk " groups must be carefully watched and the ultrasound technique lends itself to large scale monitoring, although this will only detect major vessel thrombosis. Where a number of " risk " factors are operative the early administration I^{125} fibrinogen and daily screening is undoubtedly helpful in detecting early thrombosis. Continuing research with I^{125} fibrinogen and ultrasound techniques may reveal other groups of patients at a higher risk than the average.

Once venous thrombosis has been established either clinically or diagnostically, then phlebography is indicated. This gives precise information concerning the size, location, age and adherence of the clot. Such information is essential for rational therapy (Browse *et al.*, 1967; 1969).

Both I^{125} fibrinogen and phlebography play an essential part in following the effects of therapy for thrombosis (Kakkar, 1969; Browse, 1969).

To screen all patients postoperatively or when confined to bed is at present impracticable. Thus, a large number of venous thromboses will remain " silent " until the patient develops either a pulmonary embolus or presents a few years later with changes in the lower limbs consistent with impaired venous return. However, our increased awareness of the high incidence of venous thrombosis together with the active use of these techniques should lead to some reduction in the complications arising from it.

Conclusion

At present phlebography, though the most inconvenient investigation, gives most information about an established deep venous thrombosis. I^{125} fibrinogen studies are very helpful in detecting the onset of thrombosis in high risk groups and for following the success of therapy. Ultrasound diagnosis is best used for crude screening on a large scale basis.

The other diagnostic aids described have not yet been fully evaluated clinically.

The next review will discuss the methods available for prophylaxis and therapy in deep vein thrombosis.

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News and Notices

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From January 1973 *The British Journal of Dermatology* will be published by Blackwell Scientific Publications Ltd. All correspondence concerning subscriptions should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

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Appointments

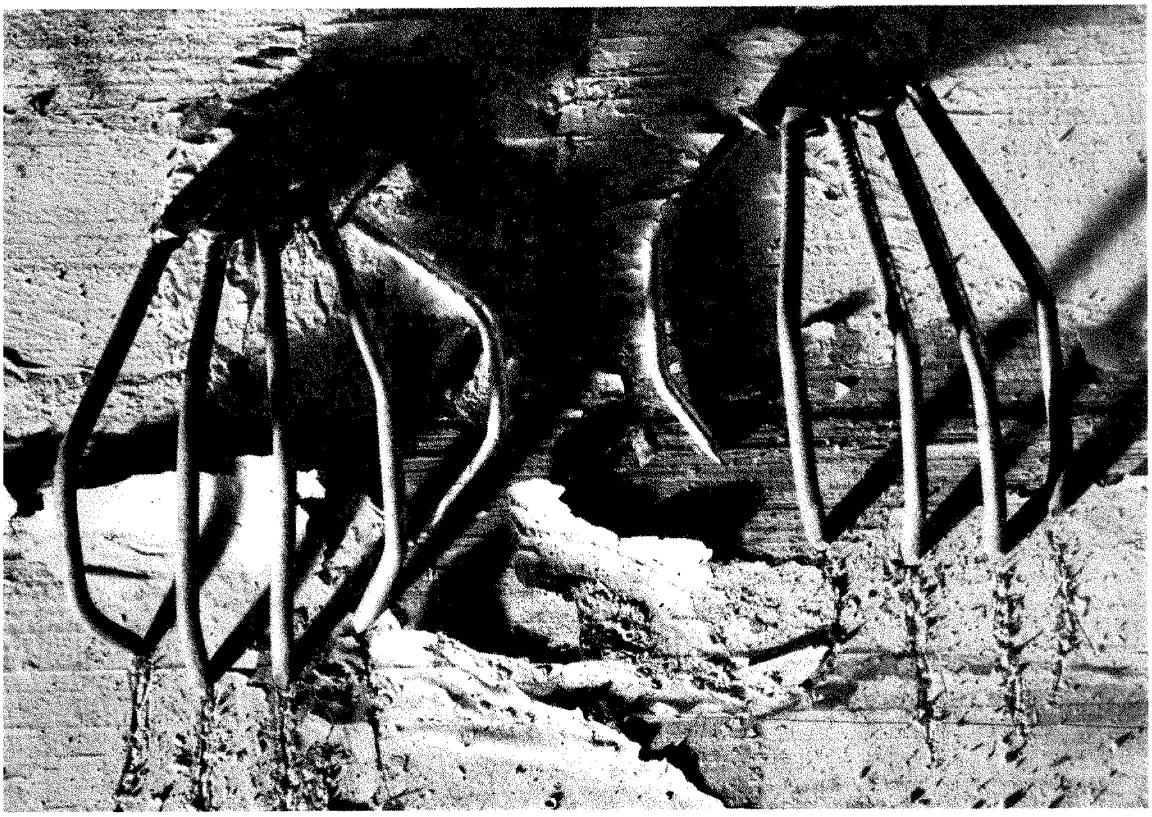
Coventry Group of Hospitals

Dr David I. Porter has been appointed Consultant Dermatologist to the Coventry Group of Hospitals, with duties initially in Coventry and Nuneaton and later in Rugby. He qualified M.B., B.S. from the London Hospital Medical College in October 1958 and he became M.R.C.P. (London) in 1962. He started in Dermatology in Newcastle with the late Professor Ingram and subsequently with Professor Shuster. He became Senior Registrar at Westminster Hospital in 1968 and eventually transferred to St John's Hospital for Diseases of the Skin. He has published a number of papers; apart from clinical Dermatology he is interested in epidermal kinetics and skin histopathology.

Correction

We regret that, in the September issue, Figure 4 (p. 194) was inverted.

Instead of just scratching the surface of the irritation problem



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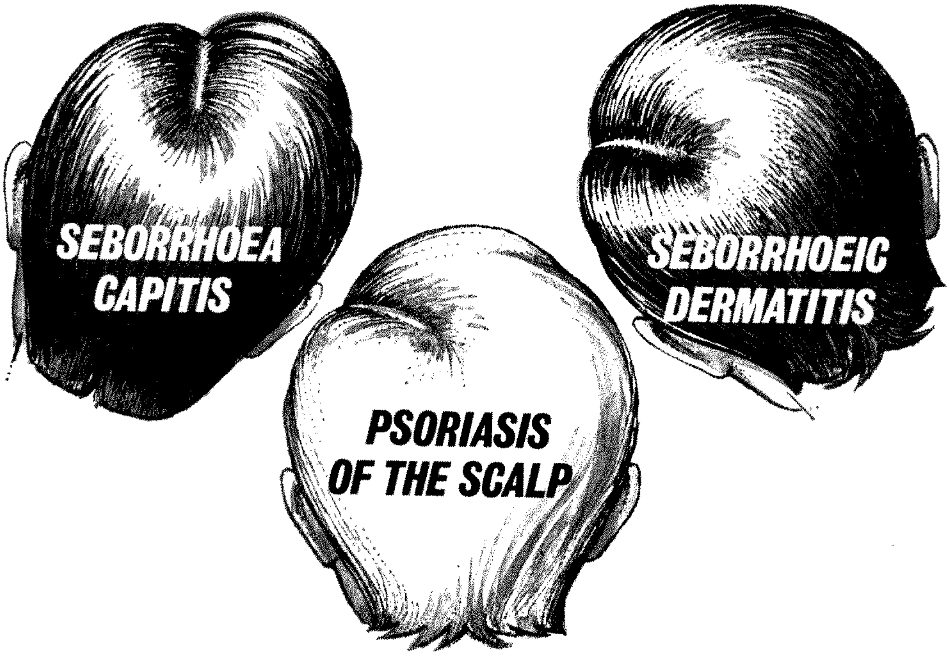

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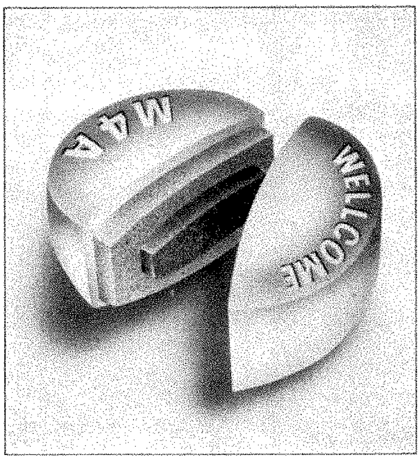

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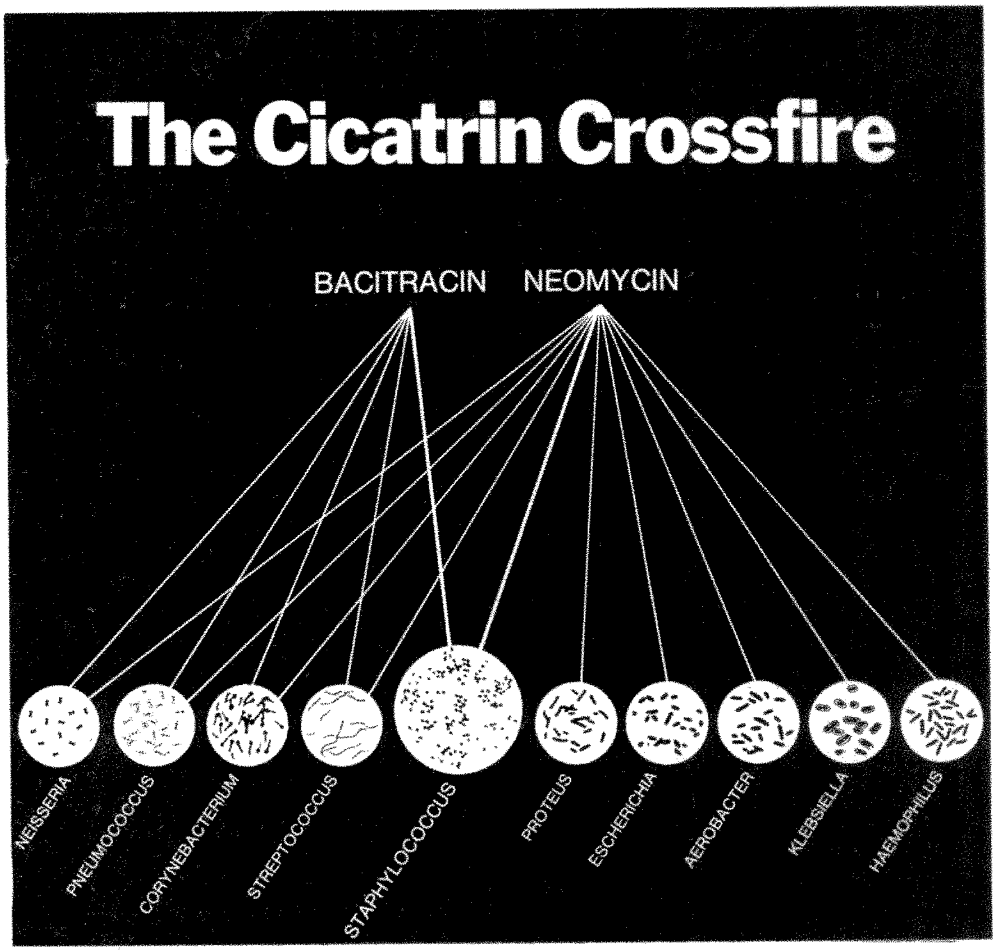

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CONTENTS (*Continued*)

Comment

Hair Replacement in the Guinea-pig and in Man
DAVID JACKSON 509

Obituary

John Thornton Ingram 1899–1972 512

Society Proceedings

Irish Dermatological Society, April 29th, 1972 514

Books and Periodicals

Protection Against Radiation from Brachytherapy Sources. Recommendations of the National Council on Radiation Protection and Measurements; Arbeitsmedizin, Sozialmedizin, Arbeitshygiene; Occupational Mycotic Infections; American Zoologist: Symposium on The Vertebrate Integument. 515

Correspondence

Separation of Stratum Corneum
A. GAFOOR and K. M. S. TOWNSEND 516
Action of Staphylococcal Epidermolytic Toxin on Mouse Skin in Organ Culture
I. SARKANY and P. M. GAYLARDE 516

Quarterly Review

Therapeutics XX. The Management of Deep Venous Thrombosis. Part I—Diagnosis
I. R. EDWARDS, ANNE E. WALKER and E. FRENK 518
News and Notices 522

BRITISH JOURNAL OF DERMATOLOGY

CONTENTS

PAGE

Clinical and Laboratory Investigations

The Natural History of Parapsoriasis en Plaques (Chronic Superficial Dermatitis) and Prereticulotic Poikiloderma
Peter D. Samman 405
Pulmonary Eosinophilia Associated with Vasculitis and Extra-vascular Granulomata. A Case Report and Review of the Literature
J. L. Burton and P. A. Burton 412
The Aetiology of Impetigo Contagiosa
M. el Zawahry, A. Abdel Aziz and M. Soliman 420
A Rosacea-like Eruption of Children
J. A. Savin, Suzanne Alexander and R. Marks 425
Perioral Dermatitis
Ian Sneddon 430
Ultrastructural Features of the Invasive Phase of *Candida albicans*
R. A. Cawson and K. C. Rajasingham 435
The "Wheat-stubble Sarcoid Granuloma": a New Epithelioid Granuloma of the Skin
J. Cortez Pimentel 444
Acid Hydrolases in the Human Epidermis
Akira Ohkawara, Kenneth M. Halprin, Joseph R. Taylor and Victor Levine 450
Comparative Study of ^{3}H-Thymidine Labelling of the Dermal Infiltrate of Skin Allergic and Irritant Patch Test Reactions in Man
J. M. Lachapelle 460
Direct Immunofluorescent Study of the Skin on Occurrence of Complement in Pemphigus
Th. Van Joost, R. H. Cormane and K. W. Pondman 466
Measurement of Lipid Synthesis in Mouse Auricular Skin Cultured *in Vitro*
C. Prottey and T. F. M. Ferguson 475

Case Reports

Unusual Features of Scabies Associated with Topical Fluorinated Steroids
A. L. Macmillan 496
Erythroderma due to Peritrate and Glyceryl Trinitrate
F. P. Ryan 498
Crohn's Disease of the Mouth
L. Stankler, S. W. B. Ewen and N. W. Kerr 501
A Case of Tinea Nigra in Britain
H. W. Chadfield and C. K. Campbell 505

(*Continued on* p. iii *of cover*)

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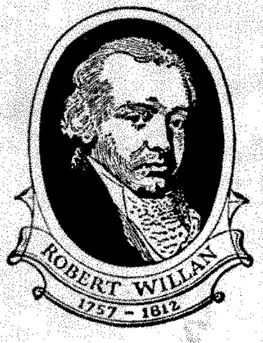

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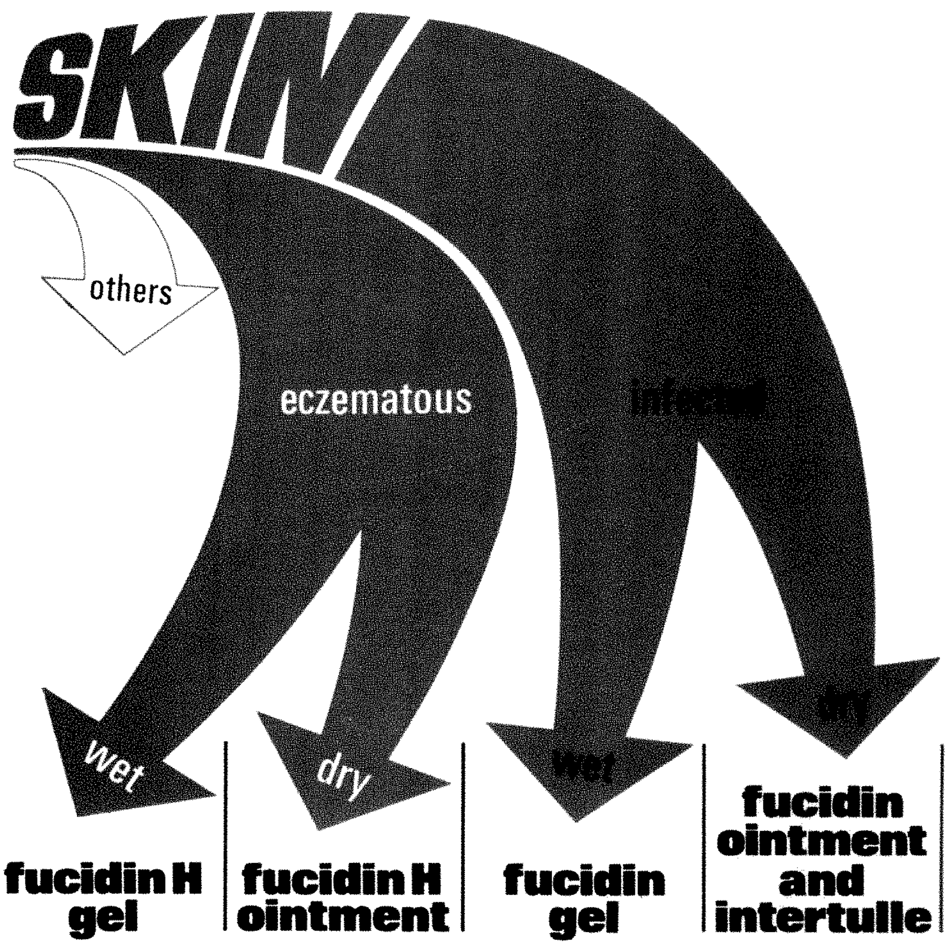

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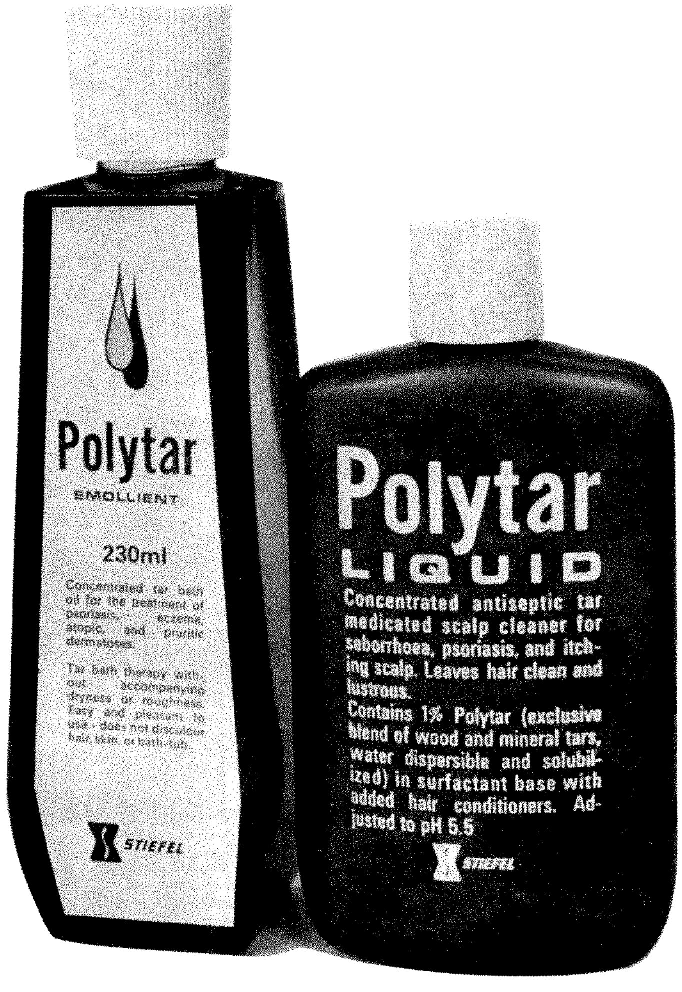
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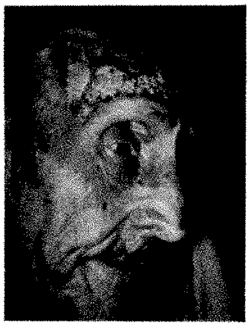

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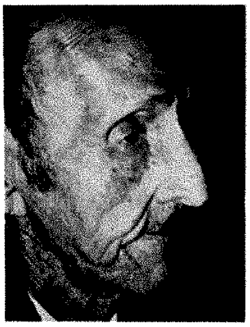

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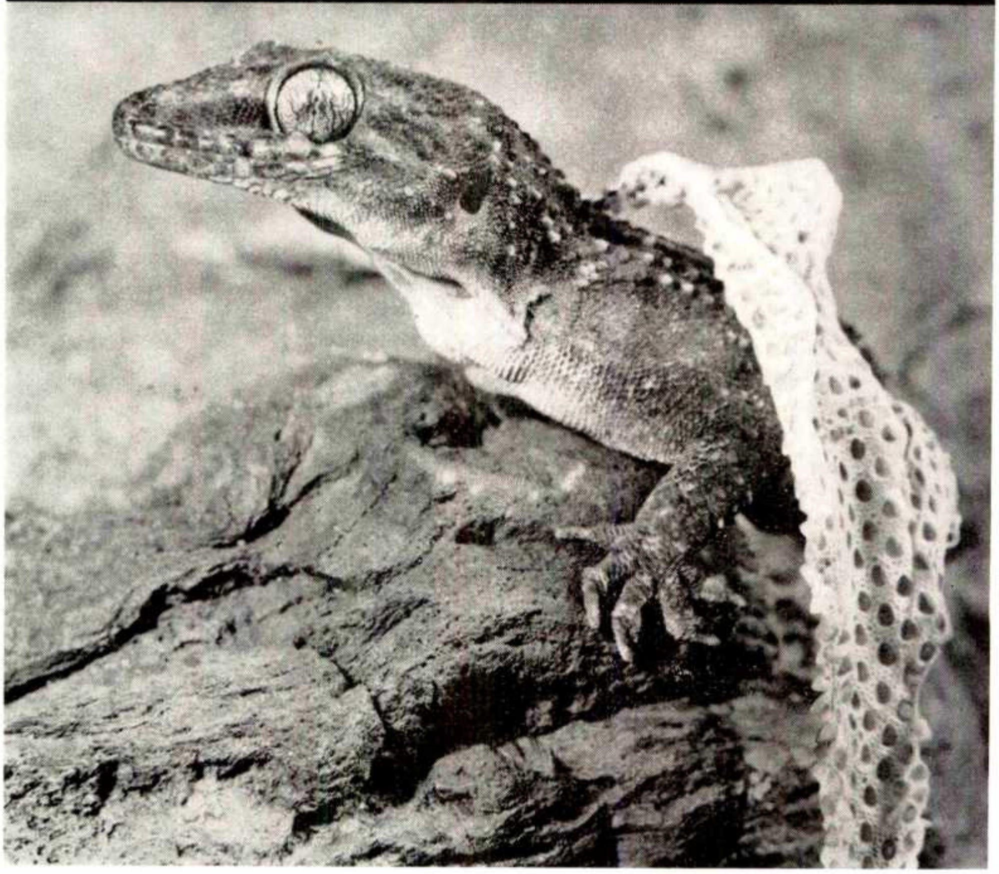

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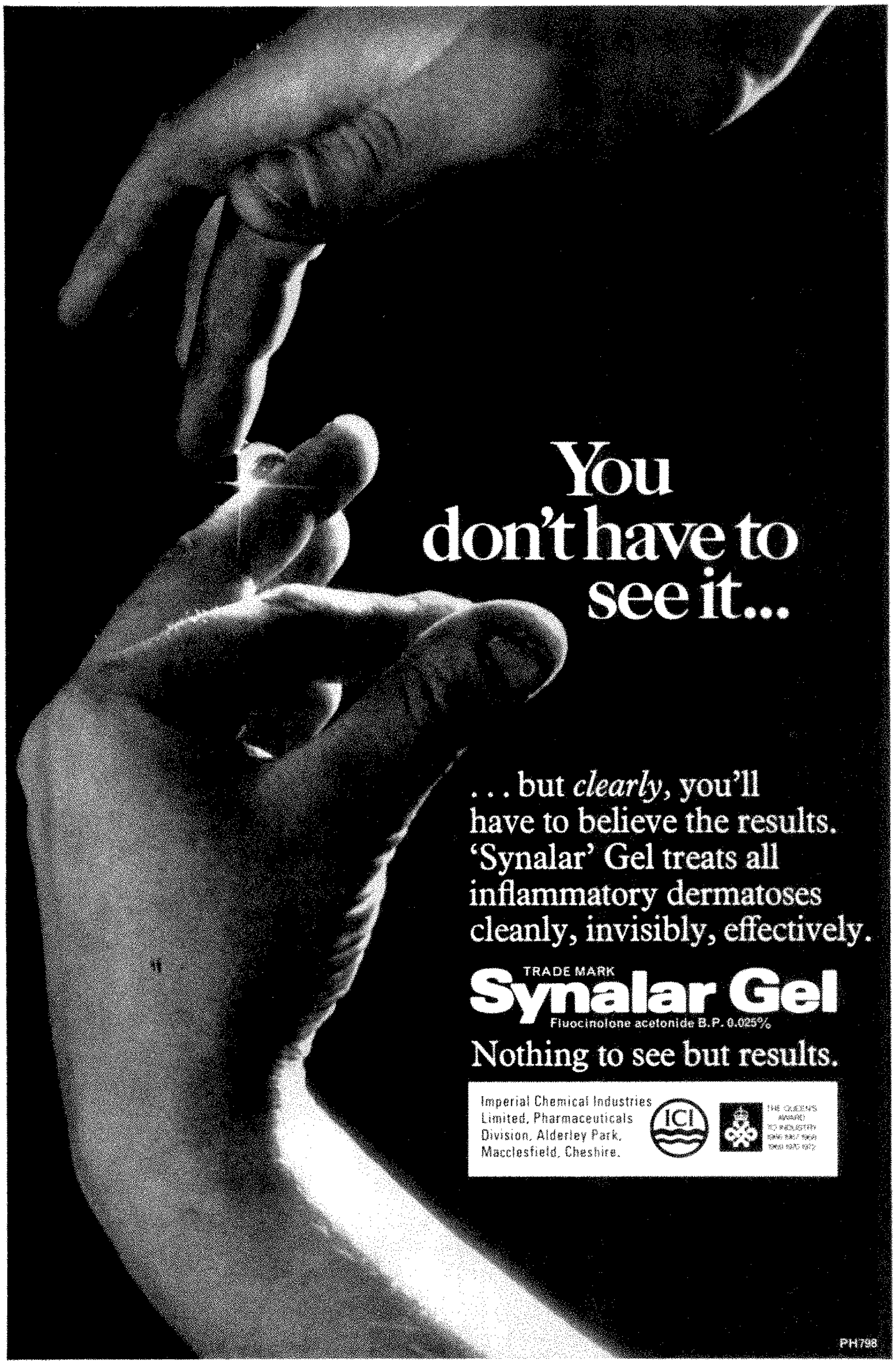

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CELL-MEDIATED IMMUNITY IN PATIENTS WITH MALIGNANT MELANOMA

R. M. MACKIE, W. G. S. SPILG, C. E. THOMAS AND A. J. COCHRAN

Summary.—The activity of cell-mediated immunity to tumour-associated antigen has been studied in 12 patients with malignant melanoma, using the leucocyte migration inhibition test. Tests carried out in 10 of these patients before surgical removal of the melanoma showed significant migration inhibition in every case. In the immediate post-operative period this inhibition was lost, and return of significant inhibition was observed in 9 of the 10 cases tested pre-operatively and in the 2 cases for whom pre-operative tests were not available. Return of leucocyte migration inhibition was noted between the 4th and 22nd post-operative day. Possible mediators of this observed depression of cell-mediated immunity are discussed.

THE possibility of immune reactions by patients against their malignant melanomata was raised in 1907 by Handley, who described round cell infiltrates around primary melanomata (Handley, 1924). He considered these to be indicators of the host natural defences against malignancy. Evidence for immunological activity against tumour tissue has accumulated rapidly in the past decade (Klein and Oettgen, 1969; Klein and Cochran, 1972), and studies of patients with malignant melanoma have proved particularly rewarding. Humoral antibody reactive to cells of malignant melanoma tissue has been reported by several workers (Lewis, 1967; Morton and Malmgren, 1968; Oettgen *et al.*, 1968; Muna *et al.*, 1969), and more recently evidence has been presented of cell-mediated immunity (C.M.I.) against melanoma (Jehn *et al.*, 1970; Nagel *et al.*, 1971; Fossatti *et al.*, 1971; Nairn, 1972; Cochran *et al.*, 1972).

In this study tumour-directed C.M.I. in patients with malignant melanoma was examined by the leucocyte migration inhibition technique (Bendixen and Søborg, 1969). This method, a modification of the macrophage migration inhibition technique (George and Vaughan, 1962), is based on the observation that sensitized lymphocytes, in the presence of specific antigen, release migration inhibition factor, which inhibits migration of leucocytes and monocytes (Søborg, 1967). During studies of leucocyte migration inhibition in 45 patients with primary and secondary malignant melanoma, variations in the degree of migration inhibition by allogeneic melanoma homogenates were observed in serial tests. This was especially noted in tests performed on cells from the same patients before and after surgery. For this reason a prospective study of tumour-directed C.M.I. has been conducted in

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12 patients with malignant melanoma who had surgical treatment. These individuals were tested at least once pre-operatively and on several occasions post-operatively.

MATERIALS AND METHODS

Patients

These were admitted for surgical treatment of clinically suspected malignant melanoma. The clinical diagnosis was confirmed in every case by histology.

Leucocyte Migration Technique

This was modified from the technique of Bendixen and Søborg. Twenty-five ml of venous blood were drawn into a syringe containing 1 ml heparin (5000 iu/ml).* The syringe was then stood vertically with the needle pointing upwards for 2 hours at 37°C. The white cell-rich component was expressed from the syringe, washed twice in saline free from calcium and magnesium ions, buffered with phosphate to a pH of 7·4 and finally with Eagle's Minimal Essential Medium with 10% foetal calf serum,† penicillin and streptomycin (MEMF). The resulting cell pellet was then resuspended in 10 times its own volume of MEMF. 200 μl samples of this suspension were then placed in 10 × 65 mm glass test tubes, the appropriate amount of antigen added and the tubes stood in air for 1 hour at 37°C. Control tubes were prepared in the same way, but contained only cells and medium.

The cells were resuspended by gentle agitation, and 4 equal aliquots drawn into capillary tubes. The tubes were sealed at one end with inert clay,‡ and then centrifuged at 600 rev/min for 4 min; the tube was then cut with a diamond at the cell-fluid interface. The cell buttons were mounted in a spot of silicone grease§ in disposable tissue culture plates|| and MEMF added to fill the plate, which was then closed with a coverslip held in place by silicone grease. Four capillaries were prepared for each test and control situation.

The complete plates were incubated in air at 37°C for 18–24 hours. The areas of cell migration were drawn with the aid of a drawing tube attached to a light microscope, and measured by planimetry. The migration area of cells pre-exposed to antigen in MEMF was compared with that of the same cells exposed only to MEMF. This ratio is the migration index.

Results obtained with the same cells in the presence and absence of antigen were compared by the Mann–Whitney–Wilcoxon U test of ranking. Significance was assessed at the 5% level.

Antigenic Extracts

Tumour samples were received at operation and placed in a sterile container. Fat and fibrous tissue were dissected away and the specimen finely chopped with a scalpel blade in Eagle's Minimal Essential Medium containing penicillin and streptomycin (MEM). The resulting material was homogenized in an MSE blade homogenizer and then spun for 30 min at 1500 rev/min at 4°C. The supernatant from these tubes was separated and its protein concentration estimated by spectrophotometry. The optimum protein concentration was established for each antigen by determining the concentration

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which maximally inhibited leucocyte migration. For the antigenic preparations used in this study optimum concentrations lay in the range of 12·5–100 μg/ml.

RESULTS

The number of tests on each of the 12 patients, the number of positive results, and the relationship of these results to surgery are illustrated in Table I. Pre-operative testing was carried out on 10 patients, and in all 10, significant inhibition of leucocyte migration occurred with at least one antigen. In the immediate post-operative period significant inhibition was not observed in any case until the fourth post-operative day when return of inhibition was noted in 2 cases. Thereafter a return of inhibition was noted in 9 further cases between the 8th and 22nd post-operative day. In one patient who had shown pre-operative leucocyte migration inhibition, inhibition was not observed at any time post-operatively.

TABLE I.—*The Frequency of Significant Inhibition of Leucocyte Migration in Patients with Malignant Melanoma Before and After Operation*

Positive reaction/antigens tested

		Post-operative (days)								
Case no.	Pre-operative	2	4	6	8	10	12	14	15	22
1	2/2		0/2		0/2			2/2		
2	—		0/1		0/1	0/1		1/1		
3	1/1	0/2		0/2			2/2		0/1	1/2
4	1/1		0/1	0/1		1/1				
5	2/2	0/2	1/2				1/2			
6	1/2	0/2						0/2		
7	3/3	0/3	1/3			1/2				
8	6/6		0/6		2/6		3/3			
9	5/6	0/6		2/4						
10	—	0/4		0/4				0/4		3/4
11	2/3		0/3		1/3					
12	2/2		0/2		0/2	1/2				

DISCUSSION

There are recent reports of depression of phytohaemagglutinin (PHA)-induced lymphocyte transformation in patients in the post-operative period (Riddle *et al.*, 1967; Park *et al.*, 1971). PHA-induced transformation is a non-specific indicator of the activity of thymus-dependent lymphocytes. The present study confirms these reports of a general depression of lymphocyte function post-operatively and additionally indicates a transient loss of specific tumour-directed cellular immunity.

It is worthy of note that in this series the one patient in whom no return of migration inhibition was observed in the post-operative period had widely disseminated disease from which she died 4 weeks after operation. Current reports on the relationship of demonstrable humoral and cell-mediated immunity to the extent of tumour progression indicate that this relationship is not well defined. Lewis *et al.* (1969) reported the loss of demonstrable humoral antibody to tumour associated antigens in patients in whom the tumour has invaded beyond the primary site and associated regional lymph nodes. In addition, Morton *et al.* (1971) reported a reduction in titre of tumour-directed antibody in melanoma patients with advanced disease. Nairn (1972), however, did not find such a stage-related variation in antibody levels.

Demonstration of stage-related variation to cell-mediated immunity in malignant disease is likewise under debate; Nagel and his colleagues (1971) have shown loss of demonstrable C.M.I. in advanced disease, as have Currie *et al.* (1971) and Van't Hoog *et al.* (1970). However, Hellstrom *et al.* (1971) and Fossatti *et al.* (1971) have not demonstrated such a relationship.

Possible mediators of the observed depression of C.M.I. are at present under investigation. Adrenal corticosteroids are known to depress delayed hypersensitivity reactions and other lymphocyte functions (Gell and Coombs, 1963). It is possible that the increased output of adrenal steroids in response to the stress of surgery is a major factor; plasma cortisol levels have been estimated in 3 of the cases presented and, in each case, a rise over pre-operative levels was noted in the 24 hours following surgery (Fig. 1). A return to pre-operative levels occurred in all 3 cases within 5 days of surgery. Leucocyte migration inhibition had returned to pre-operative levels in one of these cases by day 8, in one case by day 22, and did not return in the third case before death from melanocarcinoma. Thus, it appears from this limited study that there is no direct correlation between the duration of elevated plasma cortisol levels and depression of C.M.I., but the long-term effects of a temporary rise in plasma cortisol levels on lymphocyte function are unknown and may have considerable significance.

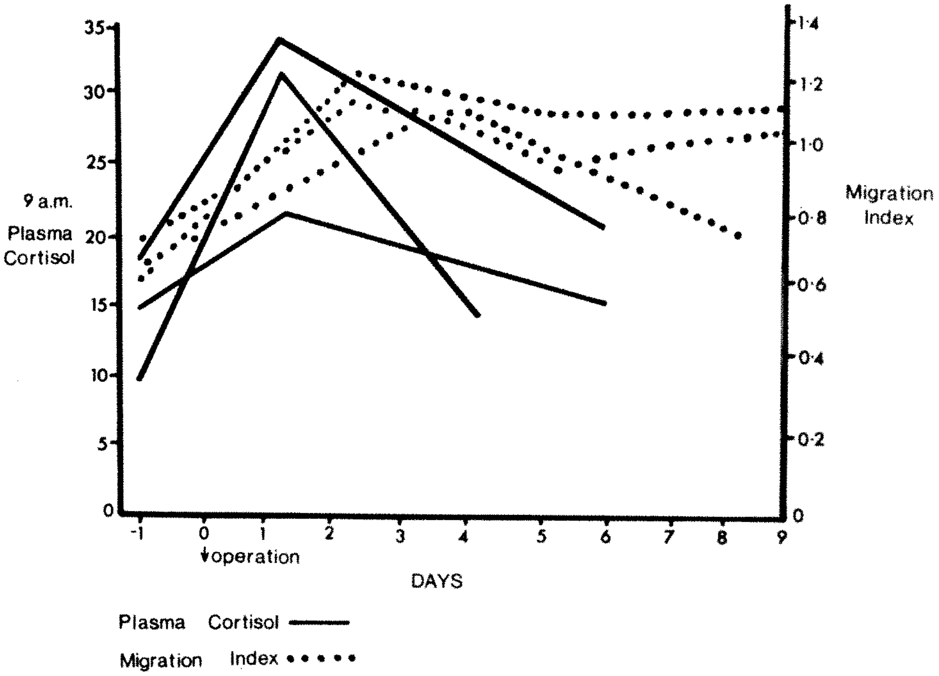

FIG. 1.—Plasma cortisols and migration indices of 3 patients in first post-operative week.

Other possible mediators of this observed depression of C.M.I. are the anaesthetic agents used at operation. It has recently been demonstrated that circulating halothane at concentrations equivalent to the upper level of those obtained during anaesthesia in man decreases the number of antibody-producing splenic lymphocytes in the rat, and that this effect is reversible (Wingard and Humphrey, 1969). In addition it has been observed that halothane has a reversible effect on lymphocyte motility (Nunn *et al.*, 1970).

In one patient studied, who had 3 successive operations at weekly intervals, tumour antigen-mediated inhibition of leucocyte migration was not detectable for a total of 4 weeks following the first operation. This finding and the general observations here reported raise important points regarding the timing of sequential operative procedures in patients with malignant melanoma. A patient with a primary lesion and clinically involved regional lymph nodes may be treated by initial wide excision of the primary site followed by dissection of involved regional nodes after an interval of 2 to 3 weeks to allow for collection within the nodes of tumour cells dislodged into the lymphatic system by operative trauma.

The findings reported in this study, if confirmed and if shown to be of clinical significance, would indicate that such a procedure is open to reappraisal.

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PYODERMA GANGRENOSUM WITH ALTERED CELLULAR IMMUNITY AND DERMONECROTIC FACTOR*

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Summary.—A patient with severe pyoderma gangrenosum, without any other associated conditions, is reported. She was found to present 2 major abnormalities of cellular immunity, i.e. lack of lymphocyte cytotoxicity induced by phytohaemagglutinin and failure of dinitrofluorobenzene sensitization. Intradermal reactions to tuberculin, trichophytin and candidin were negative, while intradermal injections of streptoccal antigen twice provoked large ulcers at the sites of injection. Dermonecrotic factor was present in her serum. High doses of steroids had to be given to heal her ulcers.

PYODERMA gangrenosum is a rare disease with recurrent deep ulceration of the skin. Brunsting *et al.* (1930) have insisted on the frequent occurrence of the lesions called by Brocq (1916–17) " phagédénisme géométrique ", with ulcerative colitis in 50–60% of cases. Since then, other conditions have also been associated: e.g. local enteritis (Stathers *et al.*, 1967); rheumatoid arthritis (Philpott *et al.*, 1966); focal sepsis (Hofstad, 1959); viral infection (Van der Meiren *et al.*, 1963); diabetes (Philpott *et al.*, 1966). More recently attention has been drawn to gammopathies (hypogammaglobulinaemia) (Karltorp, 1963); monoclonal gammopathies preceding for years multiple myeloma (Van der Sluis, 1966; Jablonska *et al.*, 1967; Thivolet *et al.*, 1969; Cream, 1971). In 1964 Long and Uesu, studying 2 cases of pyoderma gangrenosum, suggested possible troubles in humoral or cellular immunity. In the present case of pyoderma gangrenosum (already reported) (de Bast *et al.*, 1969), no associated disease occurred, but we were able to detect alterations of cellular immunity.

CASE REPORT

A woman aged 58 was in good health until 1967. At that time she developed ulcers on the legs; these lesions seemed to be related to trauma. They healed with antibacterial agents. Since then she has been readmitted 4 times for more extensive lesions on her legs, abdomen, thighs and, finally, her trunk. These lesions always began, as classically described, as sterile pustules which evolved into typical ulcerative lesions of pyoderma gangrenosum. On each occasion the lesions began to heal as soon as 60 mg prednisolone was administered daily. Her last admission in 1972 followed a skin test to streptokinase-streptodornase (SK-SD).

Investigations

All the investigations carried out to detect any related disease have been negative. E.S.R.: 2 mm in 1 hour; haematological examination: normal; electrophoresis: con-

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stantly normal; blood group: A; occult blood in stools: negative; x-ray of the whole digestive apparatus: normal; proctoscopy and rectal biopsy: normal; liver and pancreatic function: normal; no diabetes; no focal sepsis; rheumatoid factor, LE cells, LE factor, cryoglobulins: negative; Bence Jones protein: negative; x-ray of the whole skeleton: normal; urine hydroxyproline (28·3 mg/24 hours) and proline (56·3 mg/24 hours); all normal. Antistreptolysins O (ASO) were normal until May 1971. In October 1971, during a flare-up, they rose to 625 Todd units (normal value: up to 250 Todd units).

Special Investigations

All these investigations were carried out at the onset of the episodes when the patient was not receiving treatment.

Immunoelectrophoresis.—Quantitative immunoelectrophoresis, performed in 1969 and 1970 was normal. On a third occasion, in November 1971, there was a moderate increase in IgA. Serum immunoglobulin levels were measured at that time by single radial immunodiffusion on Hyland immunoplates. IgG: 1800 mg/100 ml (normal value: 1050–1670); IgA: 410 mg/100 ml (normal value: 220–360); IgM: 35 mg/100 ml (normal value: 65–105).

Direct immunofluorescence studies.—These were performed twice on samples of unaffected skin, taken near the lesions, using rabbit fluorescent anti-human globulin (Institut Pasteur, Paris), according to Thivolet and Beyvin (1967). No immunoglobulin deposit could be shown.

Indirect immunofluorescence studies.—These were performed twice with the patient's serum according to Thivolet and Beyvin (1967); no antibody was detected.

In vivo *evaluation for cell mediated immunity.*—This was studied with the following intradermal tests: Candidin (Bencard): 100 units; Tuberculin (P.P.D. Berna): 1 unit; Trichophytin (Bencard): 0·1 ml; Tetravaccine (Bencard): 0·1 ml; Streptokinase-Streptodornase (SK-SD) (Varidase Lederle): 100 units. Test sites were inspected at 15–20 min. for reaginic (weal and flare) activity and, at 24 and 48 hours, for delayed hypersensitivity. All these skin tests, twice repeated, were negative, except SK-SD and tetravaccine; these will be discussed later. Dinitrofluorobenzene (DNFB) sensitization was performed according to Turk and Waters (1969); 2,000 μg DNFB (Kodak) in acetone was applied to the skin; 15–30 days later cutaneous sensitivity, evaluated with 100 μg DNFB, was negative.

Exchange autografts.—These were carried out with a Keye's punch of 6 mm diameter, according to Long and Uesu (1964). They were taken from unaffected skin on both thighs, and sutured with nylon. One of the grafts was immersed for 15 min in a vial of Eagle's Minimal Essential Medium (Burroughs Wellcome), containing a saturated solution of hydrocortisone. Both grafts were rejected, the criterion for rejection being the formation of a crust, and redness and swelling of the surrounding skin. However, the steroid-treated fragment was rejected only after 6 days, while the untreated fragment showed signs of rejection after 2 days.

Intradermal injections of cells and sera of the patient.—These were performed according to Long and Uesu (1964). We used purified lymphocytes (up to 2×10^6 lymphocytes, 95% pure), polymorph-rich exudate, serum and plasma. All these tests were negative.

In vitro *evaluation of cell-mediated immunity.*—Lymphocytes were collected by venupuncture in plastic syringes heparinized with 10 units/ml of phenol-free heparin (Calparin). They were purified by a method described elsewhere (Delescluse and Turk, 1971).

Lymphocyte transformation.—This was assessed by culturing 2×10^6 lymphocytes/ml in 3 ml aliquots of Eagle's medium, supplemented with antibiotics, antimycotics and heat-inactivated calf serum in the presence or absence of 0·01 u/ml of phytohaemagglutinin P (PHA) (Difco). The percentage lymphocyte transformation was determined morphologically on May–Grünwald–Giemsa-stained smears after 3 days of culture; 30% or greater transformation was considered a normal response; over 50% of the cells were transformed in the 2000 cells counted on the smear.

PHA-induced lymphocyte cytotoxicity.—This was studied by the method of Perlmann *et al.* (1968), using the ^{51}Cr release of chicken red cells. Results were expressed as a percentage of the radioactivity, when stimulated with PHA. After 20 hours no cytotoxic action was observed, as compared to normal controls. The index of spontaneous toxicity was also very low, as compared to normal cytotoxic activity.

Dermonecrotic factor

To evaluate the presence of dermonecrotic factor, serum of the patient was injected into an albino guinea-pig by the technique of Ebringer *et al.* (1969a). After 6 hours a pustule appeared, soon followed by oedema and deep infiltration. After 24 hours the diameter of the lesion was 20 mm ± 3 mm. After 48 hours central necrosis occurred, and the process continued to extend up to 10 days. These ulcers strongly recalled pyoderma gangrenosum lesions. When the serum was heated at 56°C for 30 min, the reaction failed to take place, even if fresh complement was added. On 3 occasions this experiment was repeated with similar results.

DISCUSSION

Four points deserve attention:

(a) A.S.O. titres were constantly raised in each episode. However, all the intradermal tests were negative, except to tetra-vaccine and SK-SD. The reaction to these 2 was violent, giving rise to pyoderma gangrenosum.

Two hypotheses can be put forward to explain this:

(i) A specific sensitization to streptococci, causing lesions in a similar manner to that described in glomerulonephritis (Zabriskie, 1971). This case of pyoderma gangrenosum could thus be added to the list of post-streptococcal skin diseases, recently reviewed by Levene (1972), which includes vasculitis, erythema nodosum, guttate psoriasis and erythema elevatum diutinum.

(ii) A strongly positive test to streptococcal antigen following an intercurrent streptococcal infection, causing a vigorous local inflammatory reaction and giving rise to a " Köbner-like " phenomenon, as has already been well described in pyoderma gangrenosum by Piérard *et al.* (1960). This could be a concomitant phenomenon, unrelated to the pathogenesis of the disease.

(b) The rejection of autografts, delayed by steroids, as described by Long and Uesu (1964), was reproduced here; but, in contrast to their findings, auto-intradermal injections of lymphocytes failed to evoke any reaction. This rejection could result from an enhanced inflammatory response and not from a specific allergic reaction.

(c) Despite the normal *in vitro* PHA-induced lymphocyte transformation, the lack of cytotoxicity of lymphocytes and the cutaneous anergy to all allergens, even DNFB, suggest a greatly impaired cell-mediated immunity. These results can be paralleled in the recent work of Lazarus *et al.* (1972).

(d) Dermonecrotic factor is not specific for any disease and has even been reported, in weak amounts, in normal individuals. Nevertheless, to our knowledge, such a reaction, with a diameter larger than 20 mm in 10 days and with early deep necrosis, has never been reported; Ebringer *et al.* (1969b), having studied this factor, concluded that it could be a γ-globulin.

CONCLUSION

Various immunological changes have been reported in the literature in association with pyoderma gangrenosum (e.g. multiple myeloma, hypogammaglobulinaemia, gammopathies). The abnormalities reported tend to confirm that, in this case of pyoderma gangrenosum, the cellular immune system is, at least partially, incompetent. Further studies are in progress concerning the possible role of the dermonecrotic factor.

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THE INFLAMMATORY REACTION IN PITYRIASIS LICHENOIDES

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Summary.—The inflammatory component of pityriasis lichenoides has been studied with special reference to (1) the role of small blood vessels in the pathogenesis of the disorder; (2) the nature of the inflammatory infiltrate. In the course of the investigation 134 biopsy specimens were examined. Enzyme histochemistry, autoradiography and direct immunofluorescent staining were performed on several lesions, and preliminary electron-microscopic studies were made on 2 papules. No immunoglobulins or complement could be detected around small vessels by means of direct immunofluorescent techniques. The enzyme histochemical and autoradiographic results indicated that the endothelium of small blood vessels remains viable and is not focally destroyed. Enzyme histochemical and preliminary electron-microscopic data suggested that the inflammatory infiltrate is primarily histocytic in nature. It is concluded that there is no evidence that disease of the small blood vessels plays a significant role in initiating or perpetuating the disease process. It is further suggested that the primary event in the histogenesis of the lesion of PL may be the sudden massive release of vasoactive substances locally.

Pityriasis Lichenoides (PL) is a complex disorder characterized by the development of papular lesions which may become scaly or develop a purpuric component and eventually undergo necrosis. In all probability both types of lesion represent the same disease process seen proceeding at different tempos, and it does not seem justifiable to sub-divide the disease on the basis of the morphology of the lesions (Marks *et al.*, 1972). In a previous report (Marks and Black, 1972) the epidermal component of this disorder was examined with special reference to the histogenesis of the parakeratotic scale. In the present study we have investigated the inflammatory component of pityriasis lichenoides, paying particular attention to (1) the possible role of the small blood vessels in the pathogenesis of the disorder and (2) the origin and significance of the inflammatory cell infiltrate.

PATIENTS AND METHODS

All the patients studied satisfied the criteria for a diagnosis of PL as established by Marks *et al.*, 1972.

Histological and Histochemical Studies

One hundred-and-four specimens from 94 patients were available for study. Formalin-fixed and paraffin fixed and paraffin-embedded sections stained with H. and E. were examined and the site, type and density of the inflammatory infiltrate, as well as the state of the small blood vessels, were recorded.

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Papules from 14 patients with PL were removed and frozen immediately in liquid nitrogen prior to employing the following histochemical techniques:

Hydrolytic Enzymes.—(a) Non-specific esterase (indoxyl acetate-esterase method of Holt, 1954); (b) acid phosphatase (Gomori, 1941); (c) leucine aminopeptidase (utilizing 4-methoxy β-naphthylamide, Nachlas *et al.*, 1957); (d) adenosine triphosphatase (Wachstein *et al.*, 1960); (e) alkaline phosphatase (azodye method of Bancroft, 1967).

Respiratory Enzymes.—Succinic dehydrogenase; nicotine adenine dinucleotide diaphorase; glucose-6-phosphate dehydrogenase (using MBT method of Pearse, 1960).

Immunofluorescent Studies

Six papules from patients with histologically proven PL were removed and frozen immediately in liquid nitrogen before being sectioned in a cryostat at a thickness of 6 μm at a temperature of -25°C. Direct immunofluorescence was carried out by the technique described by Kay and Tuffanelli (1969). The sections were stained with fluorescein-conjugated rabbit antihuman IgG, IgA, IgM, complement (Beta 1 C) and fibrinogen (all obtained from Hyland Laboratories and diluted 1 in 10). Sections were examined under a Leitz SM fluorescent microscope with a high pressure mercury vapour lamp (HBO 200 W), using a UGI mm filter and a K 430 barrier filter.

Autoradiographic Studies

Typical scaling papules from 8 patients with PL were removed after the prior intracutaneous injection of 10 μCi of tritiated thymidine (Radiochemicals, Amersham, specific activity 26 Ci/mmol) in 0·1 ml of normal saline. Seven papules were removed after injection 50 min. to one hour previously, and one was removed after 3 days. After removal, the specimens were fixed in 10% neutral formalin, dehydrated, embedded in paraffin and routinely sectioned at 5 μm. The sections were then taken to water and dipped in Ilford nuclear emulsion (K2) after the method of Joftes and Warren (1955). Exposure at 4°C was for periods of 3, 4 and 5 weeks and the autoradiographs were developed in Kodak D 19 developer. The sections were then stained in H. and E. and routinely mounted, examined and photographed.

Electron-microscopy

Preliminary electron-microscopic studies were made on 2 fresh biopsies obtained from 2 of the patients. Small pieces were processed for transmission electron-microscopy in the usual way by fixation in 2% buffered glutaraldehyde, followed by secondary fixation in Palades buffered osmium tetroxide and dehydration before being embedded in Araldite. The sections were stained with 1·5% aqueous uranyl acetate and lead citrate (Reynolds, 1963) and examined on an AEI EM6B electron microscope, using an accelerating voltage of 60 kV.

RESULTS

Histological and Histochemical Observations

In all specimens the inflammatory cellular infiltrate was most intense in the immediate subepidermal zone and in the upper dermis. The infiltrate was for the most part arranged perivascularly, and varied in density from being relatively sparse in amount to being quite dense (see Table I). In some sections a wedge-shaped area of infiltrate extended more deeply to reach the lower dermis by sleeving a vertically arranged, small blood vessel. The infiltrate consisted of a mixture of lymphocytes and histiocytes, with a preponderance of cells of the histiocytic type (Fig. 1). In 23% a larger type of cell was present. Some of these cells were atypical in appearance in that there was a large, hyperchromatic, reniform nucleus. Other cells appeared to be binucleate. These larger cells

TABLE I

Feature	Degree	Numbers		Percentage of Total
Density of infiltrate	Sparse to moderate (+)	25	102	24
	Moderate to dense (++)	55		53
	Dense (+++)	22		21
Presence of atypical cells	—	24		23
Haemorrhage	Slight	40	46	38
	Moderate to severe	6		6
Vessel damage	Slight	17	20	15
	Moderate to severe	3		3

were found for the most part, in the dermal papillae, and were not present in large numbers—usually comprising less than 10% of the infiltrate. Oedema of the upper dermis was a prominent feature, and the small blood vessels were dilated and congested. Haemorrhage was a feature in 44% of cases; this was variable in amount, and quite often a prominent feature. In a few instances both red blood cells and smaller mononuclear cells had penetrated into the oedematous or partially necrotic epidermis above. Invasion of the epidermis by inflammatory cells occurred altogether in approximately 70%. The cellular infiltrate showed marked reactivity for the hydrolytic enzymes in many areas, especially when there was a particular predominance of larger histiocytic type of cells. The cellular infiltrate was quite strongly reactive for the mitochondrial enzymes in places, but no particular correlates of reactivity with cellular morphology were noted.

Vascular damage was not a striking feature of the biopsies examined. In 15% of biopsies in severely inflamed areas characterized by oedema, dense infiltrate and bleeding, the endothelium of some of the smaller vessels appeared blurred and swollen. In only 3% of cases was a more severe type of vascular damage found, with fibrinoid necrosis of vessel wall and leukocytoclasis. In general the small blood vessels showed the normal pattern of histochemical reactivity, with slight activity for the hydrolytic enzyme reactions and somewhat stronger activity for the mitochondrial enzymes.

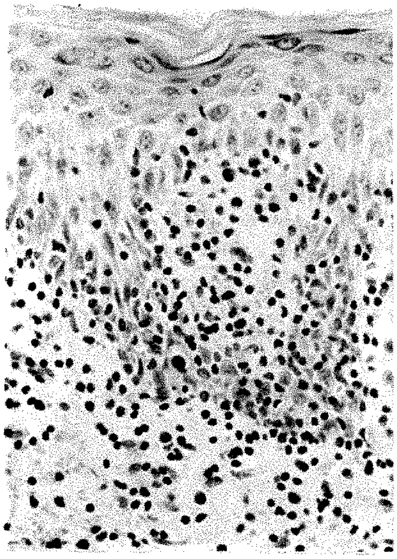


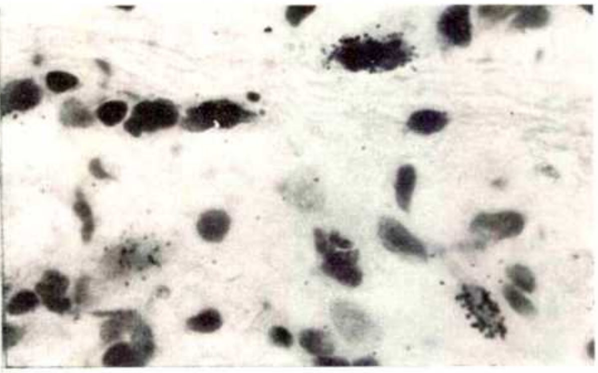
FIG. 1.—Section from lesion of pityriasis lichenoides, showing a mixed inflammatory cell infiltrate in the papillary dermis. The infiltrate is composed of several cell types; many are 2 or 3 times the size of lymphocytes and are probably histiocytic in nature. Some of the cells have large hyperchromatic nuclei and are similar to the abnormal cells of lymphomatoid papulosis. (H. and E. × 75.)

Direct Immunofluorescent Staining

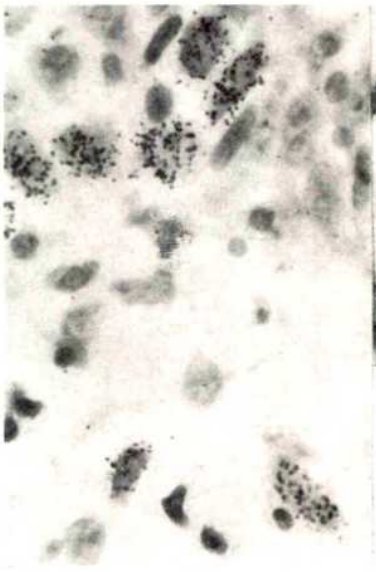
In all 6 sections examined no specific fluorescence that could be attributed to the presence of immunoglobulins or complement was detected around small blood vessels or elsewhere in the lesions. In some sections a slight granular fluorescence due to fibrinogen was seen in the dermal papillae.

Autoradiographic Observations

In the 7 specimens which had been exposed to tritiated thymidine for up to one hour prior to removal, labelled cells were scattered in the papillary dermis. In general, the more dense the infiltrate the more labelled cells that were observed. The labelled cells often seemed to be arranged in clusters or groups. Labelling was for the most part confined to the larger cells (which appeared to be histiocytes) in the infiltrate and to what appeared to be endothelial cells. In some areas labelling of endothelial cells was quite a prominent feature (Fig. 2a, 2b). A further dermal cellular element to be labelled was a spindle-shaped or crescent-shaped cell—presumably a fibroblast. The specimen removed after injection with tritiated thymidine 3 days previously also showed labelling of the larger cell types within the infiltrate—particularly endothelial cells.



(a)



(b)

FIG. 2a.—Autoradiograph from inflamed lesion of pityriasis lichenoides injected with tritiated thymidine. In the papillary dermis there are 2 labelled endothelial cells (E) (H. and E. × 150).

FIG. 2b.—Autoradiograph from lesion of pityriasis lichenoides injected with tritiated thymidine. There are several labelled cells in the infiltrate, some of which are probably endothelial cells (H. and E. × 150).

Electron-microscopy

The cells comprising the inflammatory infiltrate varied considerably in size and shape. The nuclei were often elongated and tended to be folded or indented. Most of these cells in the infiltrate contained a prominent amount of cytoplasm and appeared rich in rough endoplasmic reticulum and ribosomes, as well as containing numerous mitochondria (Fig. 3). The cytoplasm also contained vesicles and lysosomes, some of which contained a myelin-like concentric system of membranes (" myelin bodies ", see Fig. 4). These cells were identified as being histiocytic in nature.

DISCUSSION

The role of the small blood vessels in the pathogenesis of PL has for some time been the subject of controversy. In 1959 Szymanski suggested that the haemorrhage and necrosis in the lesions of pityriasis lichenoides et varioliformis acuta could be explained on the basis of a vasculitis, whereas the vascular changes in pityriasis lichenoides chronica appeared to be "astonishingly slight." Krüger and Weise (1959) noted leucocytoclastic changes in PL, and have suggested that the condition may be closely allied to allergic vasculitis. We have recently presented clinical evidence to suggest that pityriasis lichenoides et varioliformis acuta and pityriasis lichenoides chronica are merely variants of a single disease process (Marks *et al.*, 1972). In several forms of human cutaneous vasculitis immunoglobulins and complement have been seen to be deposited in or around the damaged small vessels (Scott and Rowell, 1965; Miescher *et al.*, 1965; Stringa *et al.*, 1966; Parish and Rhodes, 1967; Wemambu *et al.*, 1969; Copeman, 1970). In this investigation we have not been able to detect immunoglobulins and complement around small blood vessels in PL, suggesting that PL is not an "immune-complex" disorder, as are some varieties of vasculitis. The endothelium of small blood vessels in the centre of the lesions remained normally reactive to a range of hydrolytic and respiratory enzymes, despite being "caught up" in the inflam-

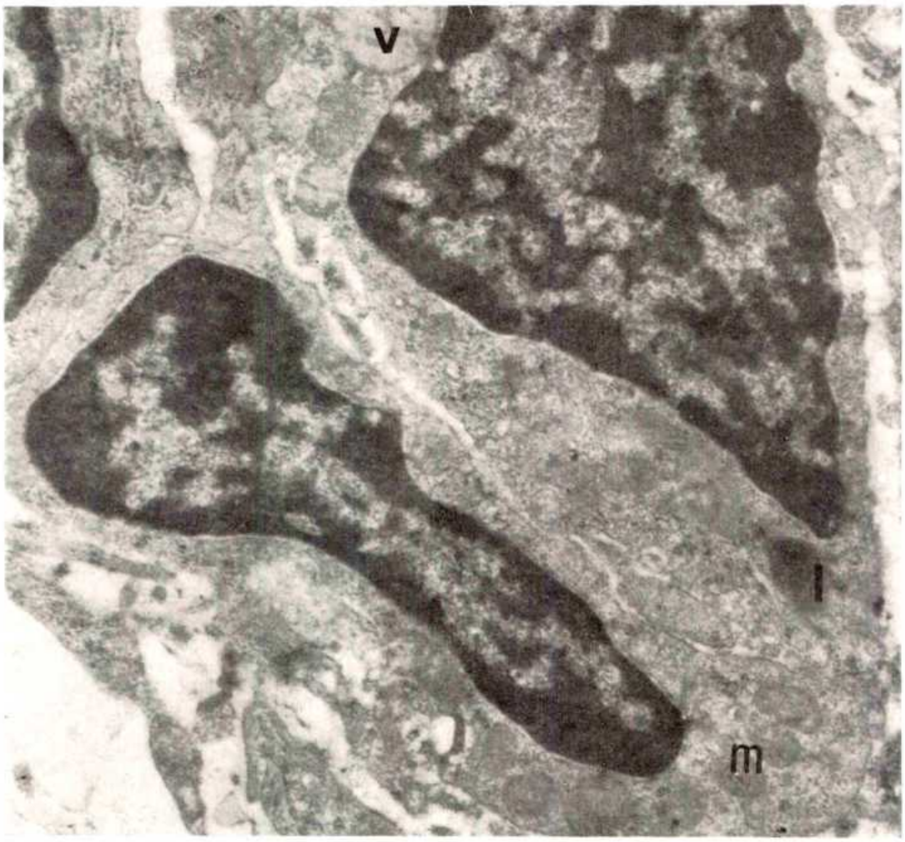


FIG. 3.—The cytoplasm of 2 inflammatory cells contain many mitochondria (m), as well as vesicles (v) and lysosomes (l). (× 10,000).

matory process. The number of labelled endothelial cells was increased in some sites to as high a level as approximately 20%, but, because of the difficulty in accurately distinguishing endothelium, it was not thought justifiable to attempt to determine an accurate labelling index. This observation also suggests that, in the majority of the lesions, the small blood vessels remain viable and capable of complex synthetic activity. For these reasons we believe that there is no evidence that the small blood vessels play any significant initiating or perpetuating role in the pathogenesis of the lesion of PL.

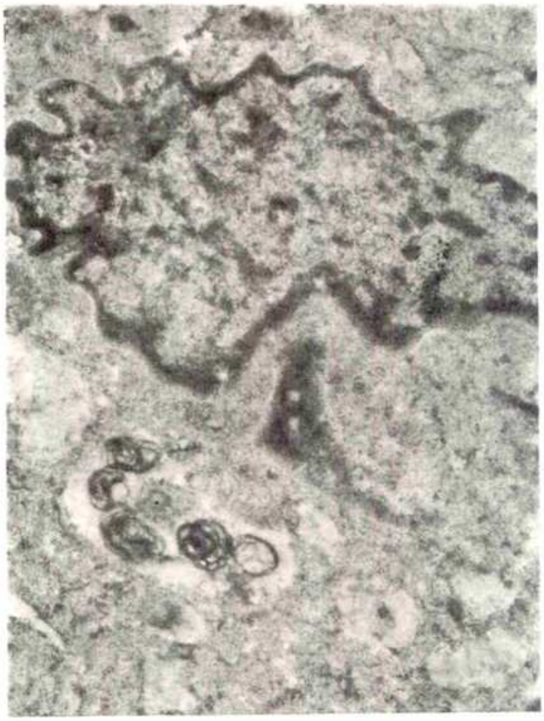


FIG. 4.—The cytoplasm of another inflammatory cell contains a " myelin body ". (× 20,000).

Enzyme histochemical and preliminary electron-microscopic data suggest that the inflammatory infiltrate in PL is likely to be primarily histiocytic in nature. Lymphocytic infiltrates show little or no hydrolytic enzyme activity (Braunstein *et al.*, 1962); but in PL papules parts of the inflammatory infiltrate appeared to be focally rich in hydrolytic enzyme activity. Electron-microscopically, the cytoplasm of the inflammatory cells was rich in cellular organelles, and some of the cells contained " myelin bodies ". Myelin bodies are lysosomal structures which can be found in macrophages of normal human skin (Breathnach, 1971). Thus the evidence suggests that these cells are in fact histiocytes.

Although the primary event in the pathogenesis of PL is still unknown, the presence of focal damage, haemorrhage and dilatation of small blood vessels in the absence of marked vacular damage suggests that the sudden release of a vasoactive material in a high concentration may play a role in the pathogenesis. The presence of a predominantly histiocytic type of infiltrate would also appear to be in favour of this concept rather than a hypersensitivity response.

We are grateful to Mr B. Bhogal and Dr C. Heather for invaluable technical assistance. R. Marks is in receipt of a grant from the Medical Research Council.

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INSULIN PRODUCTION AND GLUCOSE TOLERANCE IN PATIENTS WITH GRANULOMA ANNULARE

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Summary.—Ten patients with granuloma annulare were studied for evidence of carbohydrate intolerance. By traditional criteria, 2 had an abnormal glucose tolerance test. Of the remaining 8, 3 had an abnormal cortisone-glucose tolerance test. The findings indicate a higher incidence (50%) of glucose intolerance in these patients than is found in the average population. In the cortisone-glucose tolerance test, plasma insulin response to glucose loading was significantly lower in the population of 10 patients with granuloma annulare than in a normal control group. The remaining 4 granuloma annulare patients with normal glucose values in both the standard and cortisone-induced glucose tolerance tests were also evaluated by measuring plasma insulin response to glucose loading. These 4 patients, when evaluated as a group, demonstrated also a significantly lower insulin response to glucose than the control population. These results suggest a decreased pancreatic beta cell sensitivity to glucose stimulation in patients with granuloma annulare.

SEVERAL recent studies have suggested a relationship between granuloma annulare (GA) and diabetes mellitus (Haim *et al.*, 1970; Rhodes *et al.*, 1966; Romaine *et al.*, 1968; Wells *et al.*, 1963; Dicken *et al.*, 1969). The degree of correlation between GA and abnormalities in carbohydrate metabolism seems to be related to the intensity of investigation for diabetes. Wells and Smith (1963) found only 3 diabetics among 115 patients with GA, but their data was obtained by postal questionnaire, and no specific tests for diabetes were mentioned. Using the standard glucose tolerance and prednisone glycosuria tests, Rhodes *et al.* (1966) studied 30 patients with GA, none of whom was known to be diabetic. Two had diabetic glucose tolerance curves and 10, including the 2 diabetics, demonstrated mild carbohydrate intolerance, as measured by the prednisone glycosuria test. The results of the prednisone glycosuria test and the extent or duration of the lesions did not correlate significantly. Haim *et al.* (1970) found clinical or latent diabetes in 7 of 8 patients with generalized GA, using both the standard and the prednisone glucose tolerance tests.

Conn and Fajans (1958, 1961, 1965) showed that the administration of cortisone acetate prior to a glucose tolerance test provides a more sensitive measurement of carbohydrate tolerance and pancreatic function than the standard glucose tolerance test. Several authors (Colwell and Lein 1967; Soeldner *et al.*, 1968) have recently described an even more sensitive means of detecting carbohydrate intolerance. This is accomplished by expressing plasma insulin concentration as

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a function of plasma glucose concentration with respect to time. Roberts (1964) and Toyota and Goto (1969) considered afternoon glucose tolerance tests more sensitive than morning tests, especially when plasma insulin levels are also measured.

Because of the possible importance of GA as an indicator of carbohydrate intolerance, we studied 10 non-diabetic adults with GA and a group of normal adults as controls. Glucose tolerance tests (GTT) and cortisone-glucose tolerance tests (C-GTT) were performed in the afternoon. Both glucose and insulin levels, after an oral glucose stimulus, were measured in each of these patients. Because of the possible difference in results of afternoon tolerance tests, we compared our afternoon control subjects to previously reported data obtained in normal control subjects tested under similar conditions in the morning (Castro *et al.*, 1970). Patients with GA were chosen randomly from the University of Oregon Dermatology Clinic. The only selection involved was excluding children and overt diabetics.

METHODS

Subjects

All subjects from the normal group were healthy and ambulatory, and had no family history of diabetes mellitus. Body weight range was 100–105% of ideal weight (Metropolitan Life Insurance Table) (mean 102%). Their mean age was 24·9, and the range was 22 to 28. The 10 patients with GA were ambulatory, and ranged from 91% to 129% of ideal body weight (mean 104·9%). Their mean age was 35, with a range of 20 to 62 (Table 1). The glucose dose was 100 g in each test. The 2 challenge tests were performed at intervals exceeding 2 weeks to ensure that each test was not affected by the preceding one.

The oral glucose tolerance test and the steroid glucose tolerance test were done in the early afternoon. Considerable care was taken to employ rigorously standardized conditions including: (a) pre-test preparation with a normal caloric diet containing in excess of 300 g carbohydrate for 3 days preceding each test; (b) prohibition of any medication for the month preceding the test; (c) at least a 12-hour fasting period before each test, during which smoking was prohibited; (d) administration of 50 mg of cortisone acetate orally 8·5 and 2 hours before glucose ingestion for the cortisone-glucose tolerance test; (e) ingestion of glucose during a 5-min period in the semirecumbent position, with the subject confined to bed (Castro *et al.*, 1970) in the recumbent position for the remainder of the test; (f) constant load volume, e.g. 500 ml iced water containing the glucose and 3 g citric acid for flavouring; (g) sampling periods of 15–30 min, with an intravenous cannula to minimize trauma. Blood was drawn before glucose ingestion and 15, 30, 45, 60, 90, 120, 150, and 180 min thereafter. Serum sodium and potassium concentrations and packed cell volume were determined on all subjects at the end of each test, and were within normal limits.

Assay Methods

Venous blood was collected in a tube containing 10 u of heparin per ml of blood. Plasma was immediately separated and divided into 2 portions, one of which was frozen until insulin assays could be performed. The other was analysed promptly for glucose. Glucose was determined with the Technicon Analyzer by a ferricyanide reduction method. A sample of plasma glucose pool, which was kept frozen in small aliquots, was incorporated into each experimental run as a check on the variability of the glucose estimations; the results of the plasma pool did not vary by more than 2 mg/100 ml during the study.

TABLE I

Patients	Sex and	Age	Percentage of ideal body weight	Family history of diabetes	GA morphology and distribution	Duration	GTT results	Cortisone GTT results
A.M.	M	51	129	Negative	Annular; elbows and axillae	1·5 yr	Abnormal	—
W.I.	M	31	100	Maternal aunt	Annular: left index finger, dorsal aspect on fifth joint	1·5 yr	Abnormal	—
V.G.	M	44	106	Negative	Annular; rt. lateral arm; back of neck; hips	14 yr	Borderline	Borderline
L.D.	F	24	104	Paternal great grandfather, uncle and great aunt	Papules, often annular over extensor surface of fingers, hands, feet and elbows	11 yr	Normal	Abnormal
V.D.	F	31	91	Maternal grandfather, brother and sister	Arciform: elbows	13 yr	Normal	Abnormal
S.L.	F	21	108	Father (obese) diabetes	Arciform: left dorsal side of foot, arms and legs in past	2 yr	Normal	Abnormal
B.B.	F	38	101	Negative	Irreg. arranged papules; extensor forearms	2·5 yr	Normal	Normal
E.K.	F	62	100	Negative	Discrete and annular arrangement of papules; thighs, arms and left forearm	1 yr	Normal (flat)	Normal
C.R.	M	26	110	Negative	Annular: anterior ankle	1 month	Normal	Normal
C.S.	F	23	100	Maternal grandfather	Clear at time of test; annular; thighs and feet 6 months	1 yr	Normal	Normal

Plasma insulin concentrations were assayed using the technique of Morgan and Lazarow (1963, 1964). The I-131-bovine insulin was obtained from Abbott Laboratories, and its purity was verified before use by radioactive scanning of paper strips after electrophoresis of samples in veronal buffer and chromatography in an ethanol–water system. As a check on the variability of the insulin radioimmunoassay, a standard plasma pool sample was assayed with each run of unknown plasma insulin samples. Constant values were obtained throughout the study (32 μu/ml, S.D. = $\pm 3{\cdot}0$ μu/ml).

Clinical Features

The group of GA patients consisted of 6 women and 4 men. The duration of the disease ranged from one month to 14 years. Of the 10 patients, 9 had lesions on the extremities, and one had lesions on both trunk and extremities. Most of the lesions were flesh-coloured or were erythematous papules in an annular arrangement. The diagnosis was verified by biopsy in all cases. Study of their medical records revealed that 5 of these patients had a family history of diabetes; however, none had been previously diagnosed as a diabetic or carbohydrate intolerant (Table I).

Analysis of Results

The Standard American Diabetic Association criteria were used in determining normal glucose values in the standard glucose tolerance test. We used Fajan's and Conn's criteria for normal glucose values in the cortisone-induced glucose tolerance test. Since glucose and insulin determinations were done on plasma, conversions to blood glucose values were necessary to compare our values with the standard criteria mentioned above; 15% was subtracted from each plasma glucose value to arrive at a comparable blood glucose value. Blood glucose values had to be elevated at least 10% above standard values to be considered " abnormal ". Lesser elevations at critical times during either test were considered " borderline ". To analyse the relationship between plasma glucose and plasma insulin levels during both the glucose tolerance test and the cortisone-glucose tolerance test, we calculated the ratio of insulin to glucose levels with respect to time in each patient and normal control. These ratios were averaged within 3 separate groups: (1) normal controls; (2) GA patients (all 10 in GTT and 8 without diabetes in C-GTT); (3) GA patients with seemingly normal glucose tolerance in both the GTT and C-GTT. For clarity in graphical representation of the groups, regression analysis was used to find the best straight line through the mean ratio values at each time. Each dot on the graph in Fig. 3, 4 represents a mean glucose *vs.* insulin level of a population at a given time after glucose ingestion. According to the work of Colwell and Lein (1967) and Soeldner *et al.* (1968), the steeper the slope or the further the line is shifted to the left, the greater the insulin response to glucose. Conversely, the flatter the line and the more the line is shifted to the right, the lower the insulin response to glucose. Using these insulin/glucose ratios, our GA patients were compared to our normal control subjects in both the glucose tolerance test and the cortisone-induced tolerance test, using coefficients of correlation for statistical analysis.

RESULTS

The results of both the standard GTT and C-GTT during the afternoon tolerances in our normal control subjects were well within the normal range for glucose results (American Diabetes Association criteria; Fajans and Conn, 1958, 1961). We also found their average insulin and glucose results to be no different from the levels previously reported in normal control individuals after morning tolerances (Castro *et al.*, 1970).

The initial glucose tolerance test performed on the 10 GA patients revealed that 2 of the subjects had abnormal glucose results. Both of these patients demon-

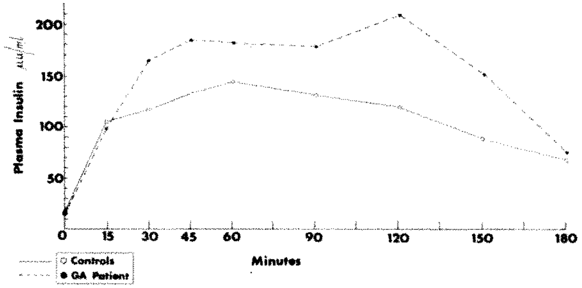

FIG. 1.—Plasma insulin levels during the standard glucose tolerance test.

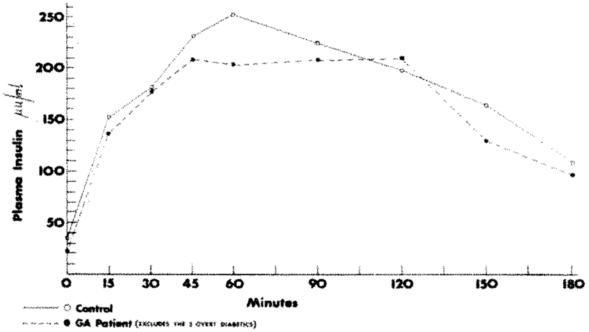

FIG. 2.—Plasma insulin levels during the cortisone glucose tolerance test.

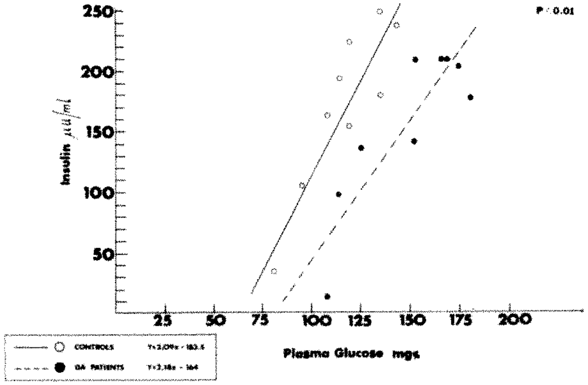

FIG. 3.—Mean regression lines of insulin response to glucose on C-GTT. Normal controls *vs.* all GA patients(8) except overt diabetics(2).

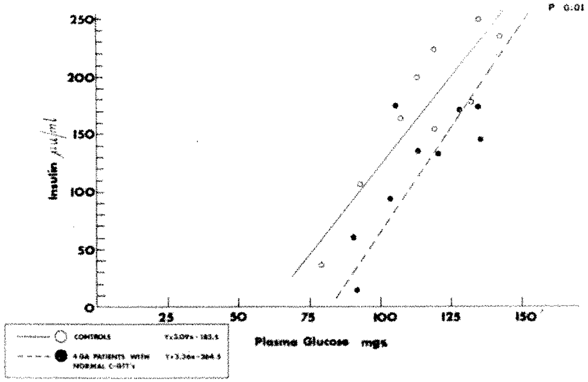

FIG. 4.—Mean regression lines of insulin response to glucose on C-GTT. Normal controls *vs.* 4GA patients with normal glucose levels on GTT and C-GTT.

strated glucose values above those considered normal, using the American Diabetes Association criteria. An elevated but latent immuno-reactive insulin response was also observed in both patients, further confirming a diagnosis of maturity onset diabetes (Bagdade *et al.*, 1966; Yalow *et al.*, 1965).

More extensive investigation was completed on the remaining 8 granuloma annulare patients, using the cortisone-stimulated glucose tolerance test. Three patients were shown to have abnormal glucose tolerance in this test, and one was borderline, using the criteria established by Fajans and Conn (1958, 1961). Thus, 5 of the 10 granuloma annulare patients studied had evidence of abnormal glucose tolerance; one was borderline, and 4 were seemingly normal glucose-tolerant patients, as measured by glucose value alone during the GTT and the C-GTT.

Since plasma insulin levels have not been as well standardized as glucose levels, we have expressed mean insulin levels graphically for visual comparisons without considering them to be normal or abnormal. The GA population as a group demonstrates a greater but delayed insulin secretion than the controls in a standard GTT (Fig. 1). However, after a more severe challenge produced by the C-GTT, the GA patients (excluding the 2 maturity onset diabetics) showed a slightly decreased insulin response when compared to the controls (Fig. 2). Again, the latent insulin response peak is observed in the GA patients during the C-GTT.

Using regression analysis to find the coefficients of correlation, the granuloma annulare patients as a whole were compared to the normal control subjects with respect to their insulin/glucose ratio. The results of this comparison showed little difference between these 2 groups in the standard glucose tolerance test. However, in the C-GTT there was a significantly lower insulin response to glucose levels ($P < 0{\cdot}01$) in the 8 GA patients as compared to the controls (Fig. 3). The population of 4 GA patients with normal glucose results (in both the standard and C-GTT) also demonstrated less ($P < 0{\cdot}01$) insulin response to glucose than controls in the C-GTT (Fig. 4).

DISCUSSION

Several authors have described a generalized type of granuloma annulare (Haim *et al.*, 1970; Romaine *et al.*, 1968; Dicken *et al.*, 1969). Whereas the generalized type usually occurs in patients over 50, the typical, more localized type usually affects patients less than 30 years of age. Haim postulated that the 2 forms may have a different pathogenesis and that the generalized type, at least, is closely related to diabetes. We have not been able to divide our patients easily into morphological groups because, in several cases, the disease is neither localized nor generalized, but somewhere in between (Table 1). We have not been able to correlate from our data glucose intolerance with morphology or with extensiveness of the GA. However, our sample size is small, and firm conclusions cannot be drawn from these patients alone.

We have noted earlier that the mean age of our GA patients is 10 years greater than that of the controls. Results of studies correlating age with insulin levels are contradictory (Kilbridge *et al.*, 1968; Chlouverakis *et al.*, 1967; Boyns *et al.*, 1969). Possibly the decrease in insulin response to glucose in our patients with GA may be due in part to their being a slightly older group. We doubt that age is a significant factor, however, because our results are similar when using only our first 8 patients (excluding patients 1 and 8 in Table I). Their average age is 30, only 5 years older than that of the controls.

Soeldner *et al.* (1968) asserted that the glucose/insulin relationship is more sensitive in detecting mild carbohydrate intolerance than glucose values only during the cortisone-induced glucose tolerance test. By using insulin/glucose ratios during the cortisone GTT we have shown the GA patients as a whole to have a considerably lower insulin response to glucose than our normal control subjects (Fig. 3). Even more interesting are the insulin/glucose ratios during the C-GTT of the GA patients demonstrating normal glucose results (Fig. IV). This group also differs from our control subjects by demonstrating a lower insulin response to glucose. Although these findings suggest that some patients with GA have a carbohydrate intolerance too low to be detected by standard glucose tolerance and cortisone-induced glucose tolerance tests, larger studies are needed for confirmation. Nevertheless 5 (50%) of our GA patients demonstrate carbohydrate intolerance by standard techniques, an additional patient having borderline glucose values. Further, our data suggest that, by using more sensitive techniques (insulin/glucose ratios during the C-GTT), an even greater percentage of patients with GA have reduced carbohydrate intolerance as compared to controls (Fig. 4).

Our study confirms other reports of a significantly greater incidence of glucose intolerance in granuloma annulare patients than in the general population. As one might expect, the percentage of granuloma annulare patients with carbohydrate intolerance increases as the method for detection becomes more sensitive.

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CORTICOSTEROID-INDUCED ATROPHY OF THE SKIN WITH TELANGIECTASIA

A CLINICAL AND EXPERIMENTAL STUDY

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Summary.—Six patients with corticosteroid-induced atrophy of the skin with telangiectasia are recorded and are briefly discussed. The skin changes were produced experimentally in 3 volunteers by prolonged topical application of " Synalar forte " and studied with light and electron-microscopy. It is suggested that the first changes in the dermal tissue occur in the ground substance, followed by those of elastic and collagen fibres. These changes are ascribed mainly to the incomplete inhibition of fibroblasts by the corticosteroid.

CORTICOSTEROID-INDUCED atrophy, relatively recently reported, is usually observed on the face and hands and is produced mainly by fluorinated steroids. The histological investigation of the condition is complicated by the solar elastotic changes inevitably present in these sites.

To our knowledge there has been no experimental approach to this problem in man duplicating the procedure which induced the iatrogenic atrophy.

CASE REPORTS

The main features of our 6 patients observed in the course of the last 2 years are summarized in Table I.

TABLE I

Case	Sex and age	Type of corticosteroid	Duration of treatment	Diagnosis and site	Histological examination of atrophy
1	M 31	Difluorinated	9 months	Tinea Face	Yes
2	F 13	Difluorinated	8 months	Tinea Face	Yes
3	F 32	Nonfluorinated hydrocortisone	Several years	As a cosmetic Upper lids	No
4	M 43	Difluorinated	18 months occlusive	Dorsa of hands	Yes
5	F 18	Mono and difluorinated	Several years	Atopic derm. Face	No
6	F 10	Difluorinated	Several years	Atopic derm. Face	No

Comment.—The clinical features in all patients were telangiectasia scattered on atrophic skin. The skin was smooth and relatively featureless, and finely wrinkled. There was increased pigmentation in 2 patients (1, 3); in one of them (1) hair growth was increased on the treated side. One patient suffering from palmar pustulosis (4) applied corticosteroid ointment also on the dorsa of hands, on his own initiative.

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Histopathological examination.—This revealed a moderately atrophic epidermis and signs of actinic degeneration. Among the orceinophilic material, linear, pseudo-colloid-like or multiform in appearance, narrower intact collagen bundles were present. The skin adnexae were of normal appearance. There was a sparse round cell infiltrate situated mainly around the blood vessels. Fibroblasts in moderate number, oval or elongated in shape, with hyperchromatic nuclei and barely visible cytoplasm, were present throughout the dermis. Mastocytes were rarely seen. Electronmicroscopic examination showed solar elastotic changes. Among the intact collagen bundles, granular or more structureless material, more or less electron-dense, of irregular appearance, was seen. Some rare fibres showed longitudinal brush-like splitting.

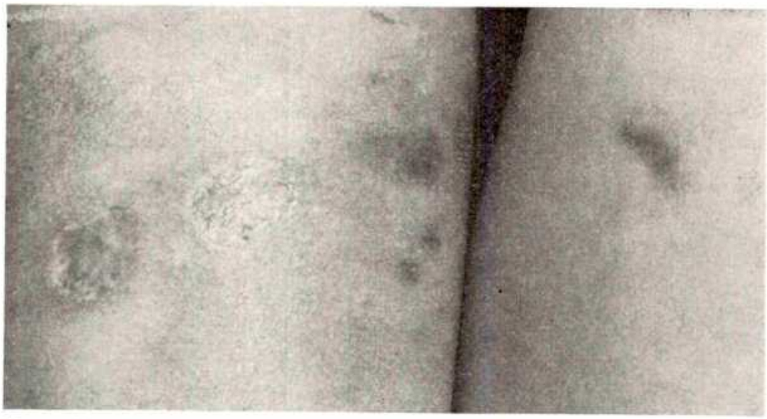

FIG. 1.—Unusually wide biopsy scars are depressed below the level of the surrounding experimental surface, which is covered by telangiectasia. The hypertrophic scar of the untreated contralateral control side is also shown.

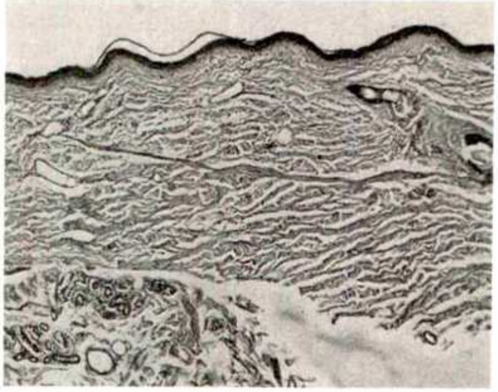

FIG. 2.—The general histological appearance of the corticosteroid induced atrophy.

Experimental investigation.—Synalar forte (fluocinolone acetonide 0·2%) was applied on the flexor aspect of the forearm under permanent occlusion on an area 6 cm square for 3–4 months. The volunteers were 3 normal male adults aged 30 to 40. After this time, discrete atrophy of the skin, with distinct telangiectasia was present. Lanugo hairs were not apparently coarser or more numerous than in the area surrounding the experimental surface (Fig. 1).

Biopsies of these sites were carried out and the material was microscopically and ultramicroscopically examined.

The biopsy wounds, which became unusually wide, continued to be treated with "Synalar forte", as was the rest of the experimental surface. After 4–5 weeks, the biopsy wounds healed with a superficial, slightly depressed scar. The control biopsy scar of the opposite forearm was slightly hypertrophic (Fig. 1).

RESULTS

Microscopic examination.—A clear cut atrophy of the whole skin was present. The epidermis was reduced to 3–4 layers of cells. There was mild hyperkeratosis. Clear cells were easily distinguished and were not obviously reduced in number.

The depth of the dermis was reduced; collagen bundles were shrunken, homogenized and separated by spaces (Fig. 2). The underlying fat was of normal appearance. Within the mid and deeper dermis, elastic fibres were coarser; short rods and cubes were often seen; in the papillary zone they were finer and longer. Orceinophilic material suggesting solar elastotic changes was not present. Blood vessels were dilated, often with swollen endothelial cells. The elastic lamina of the walls of deeper vessels was continuous. A space separated the upper dermal vessels from the surrounding collagen and elastic fibres. Sebaceous glands showed no more vacuoles in their cytoplasm than are usually seen in paraffin sections. The coil and secretory portions of the eccrine glands appeared

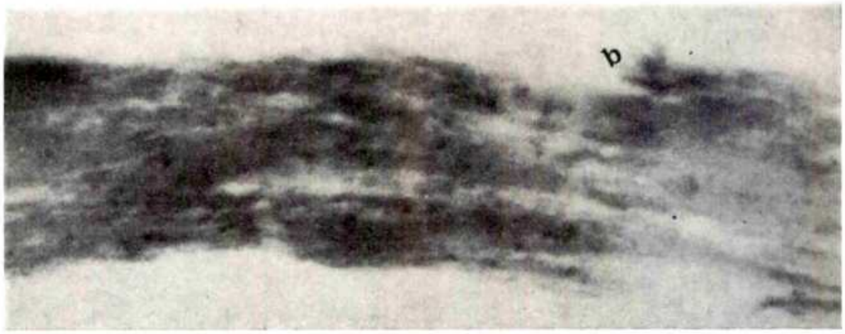

FIG. 3.—An elastic fibre in the process of degeneration, exhibiting splitting. Skeleton fibres have disappeared, while the dense matrix is unequally distributed over the fibre—at some places missing completely. There is a suggestion of beading of one "split fibril" (b). (UA—lead citrate × 36,000.)

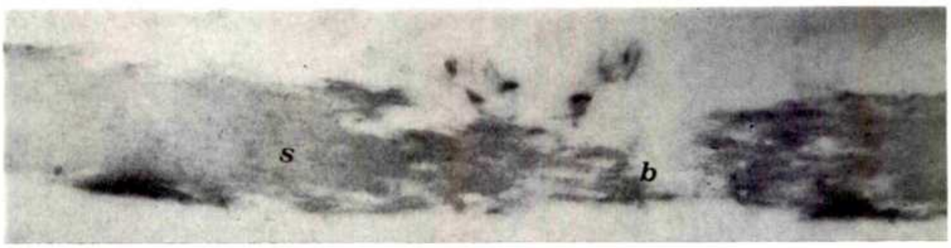

FIG. 4.—Nearing its breakage point, elastic fibre becomes split. Three "fibril-like splits" exhibit beading (b). A few skeleton fibres (s) are also present. (UA—lead citrate × 14,700.)

normal; with PAS, glycogen could be demonstrated within them. In one patient, however, the cells, mainly those of the secretory portion also containing glycogen, were mostly detached from the basement membrane and arranged in a disorderly fashion within the lumen. The walls of the secretory segments with the myoepithelial cells attached, seemed to be distended (Fig. 2). The continuity of the orceinophilic wall was usually preserved; rarely it was longitudinally split. Two hair follicles, with their arrector muscles, were of normal appearance. A round cell infiltrate, mainly perivascular, was sparse. The lymphocytes and histiocytes which composed it were of normal appearance. Fibroblasts were scattered throughout the dermis, apparently not reduced in number. They were round, oval or, rarely, fusiform in shape, often not situated along the collagen bundles. Their hyperchromatic nuclei occupied nearly the whole cytoplasm, its paler rim being rarely discernible, and only then with difficulty. With Alcian-blue-PAS stain, no depolymerization within the dermis, or any other change, was noticed.

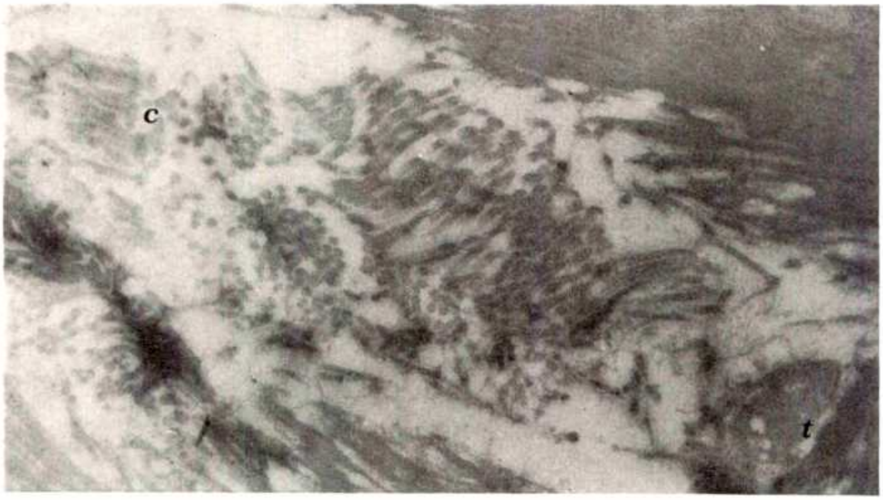

FIG. 5.—Three elastic fibres in different stages of degeneration. The upper one is hardly changed and the lower one is split into "fibrils" among which less dense amorphous material is present. The tangentially cut elastic fibre (t) consists of amorphous material within which empty spaces are present. Among the elastic fibres, collagen fibres (c) with visible striation are present. (UA—lead citrate × 16,000.)

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Ultrastructural changes.—Practically all the elastic fibres showed more or less pronounced signs of degeneration. Skeleton fibres were less recognizable, or had completely disappeared. The dense matrix was often paler, the rarification not always being equally distributed over the whole affected fibre (Fig. 3). Complete breakage of a fibre was also seen (Fig. 4), and longitudinal splitting to give a brush-like appearance, was not uncommon (Fig. 5). The complete disappearance of dense matrix, leaving only the peripheral dense zone, was also seen (Fig. 6). Collagen fibres of uniform size and with typical striation were situated among such altered elastic fibres. The close proximity of normal-appearing collagen fibrils to their merging with a degenerative elastic fibre was also recorded (Fig. 7). The spaces between the collagen fibres forming a bundle were irregular (Figs. 8, 9). Collagen fibres pertaining to different bundles were of the same diameter. On sections, fibroblasts were round or oval in shape; their hyperchromatic oval- or kidney-shaped nuclei filled nearly the whole of the central part of the cell body (Fig. 6). The short cytoplasmic processes contained organelles as well as smaller cysternae lined by ribonucleoprotein particles. A tropocollagen–precollagen complex adjacent to the plasma membrane of a fibroblast was also seen.

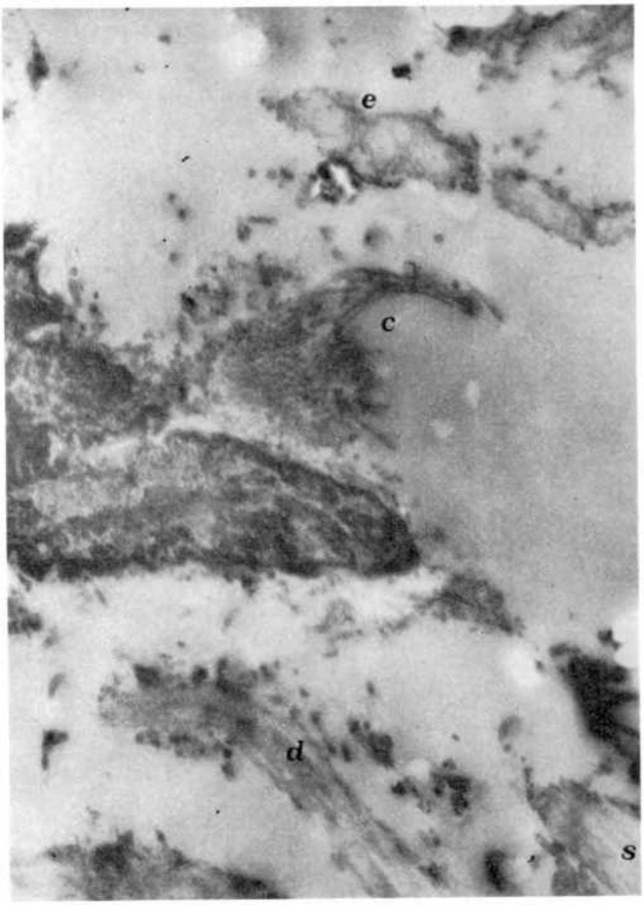

FIG. 6.—A section through a fibroblast with tropocollagen-precollagen complex adjacent to its plasma membrane. Above a collagen bundle (c), and a nearly completely vaculoated elastic fibre (e), with well preserved peripheral zone. A part of another less degenerate elastic fibre (d) is also seen. On a further elastic fibre, skeleton fibres are also visible (s). (UA—lead citrate × 12,000.)

DISCUSSION

Both the clinical and the experimental picture of atrophy with telangiectasia differed from senile skin. The skin could be wrinkled in "cigarette paper" folds. Its surface was smooth and shiny, and the follicular orifices were less visible. In addition, the experimental surface was paler and whiter, and depressed below the surrounding skin. Thus the term " cutis presenilis medicamentosa ", as used by some authors, may not be entirely appropriate.

Atrophy with telangiectasia induced by a nonfluorinated corticosteroid (Patient 3) can be best explained by its very prolonged use and the special microanatomical features of infected skin.

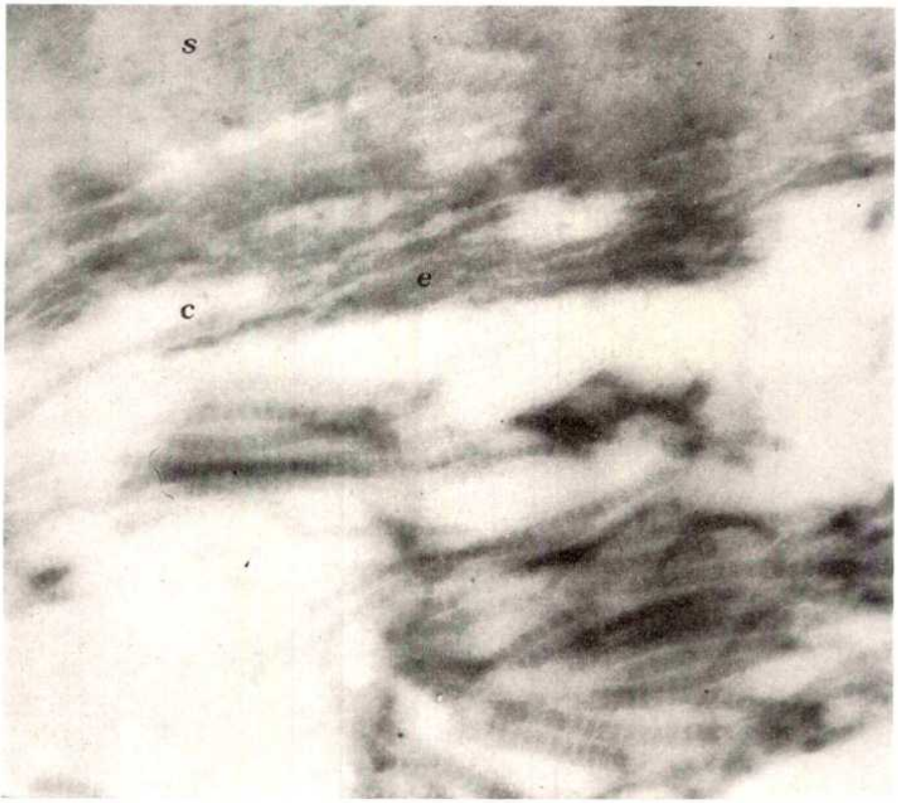

FIG. 7.—A normally-striated collagen fibre (c) is closely attached to part of a split elastic fibre (e). On the elastic fibre only a few skeleton fibres (s) are recognizable. (UA—lead citrate ×32,000.)

Although the hairs on the treated side of the first patient appeared to become slightly coarser and more numerous, no such changes were noted in other patients or in the experimental subjects.

The sebaceous glands seen did not seem to be unusually large but they were small and scanty at the experimental sites. Enlarged sebaceous glands in patients receiving systemic corticosteroid therapy are known as " cutis linearis colli punctata ". The wall of eccrine glands in one of our subjects showed definite expansion.

The most conspicuous ultramicroscopic changes seen after the experimental period we selected were therefore those of the elastic fibres; less striking were those of collagen. In mature elastic fibres the skeleton seemed to be first affected, followed by the dense matrix. The intensity and spread of changes on both elastic and collagen fibres could, however, hardly account for the gross clinical appearance.

In many investigations dealing with the effect of corticosteroids on the skin, biochemical analysis of hydroxyproline (and less often of hexosamine) has been carried out. The authors have not, however, been unanimous in their findings.

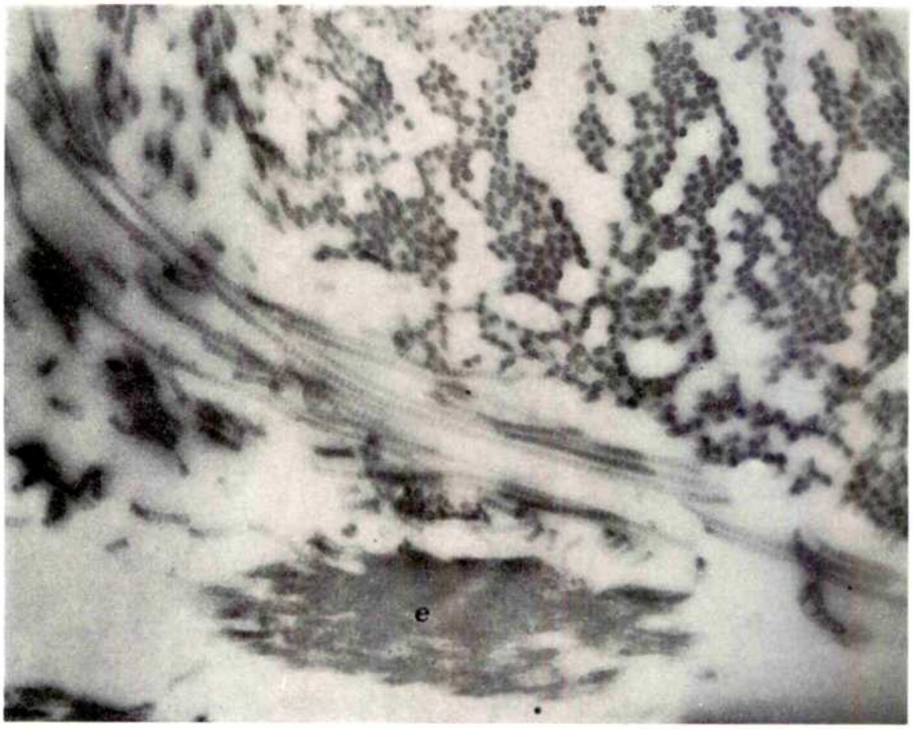

FIG. 8.—Within a collagen bundle, fibres are unequally distributed among " empty spaces ". The contiguous, longitudinally positioned collagen fibres show normal striation. The elastic fibre (e) shows changes similar to those described in Fig. 7. (UA—lead citrate × 20,500.)

A reduction in the volume of collagen fibres in rats under the topical effect of " adrenocortical hormone " was seen histologically by Castor and Baker (1950); elastic fibres were apparently not affected. Baxter *et al.* (1951) came to similar conclusions. Nimni and Bavetta (1964) studied the influence of methylprednisolone (by intra-peritoneal injection of glycine-C^{14}) on collagen synthesis and turnover in rats. They concluded that steroids inhibit collagen synthesis, but also retard its breakdown. Also using radioactive glycine, Smith and Allison (1965) found that cortisone acetate primarily decreases synthesis and does not increase degradation of collagen. Harris and Sjoerdsma (1966) described a " collagen profile ", measuring soluble, cross-linked and total collagen in patients rendered cushingoid by prolonged corticosteroid treatment. In all patients " the profile " was judged as normal. In patients who had received prednisone therapy for 2 weeks, however, Lorenzen and Zachariae (1966) found a reduction in the hexosamine content of the skin.

The findings on the effect of corticosteroids on dermal spread are often contradictory (Stary, 1963), but the inconsistencies may partially reflect different experimental conditions as well as the animal species used.

Normal ground substance, because of its amorphous state and low optical density, is not discernible under the electronmicroscope. Possible altered and visible ground substance was not seen by us; nor was there depolymerization which could be detected by the histochemical methods used.

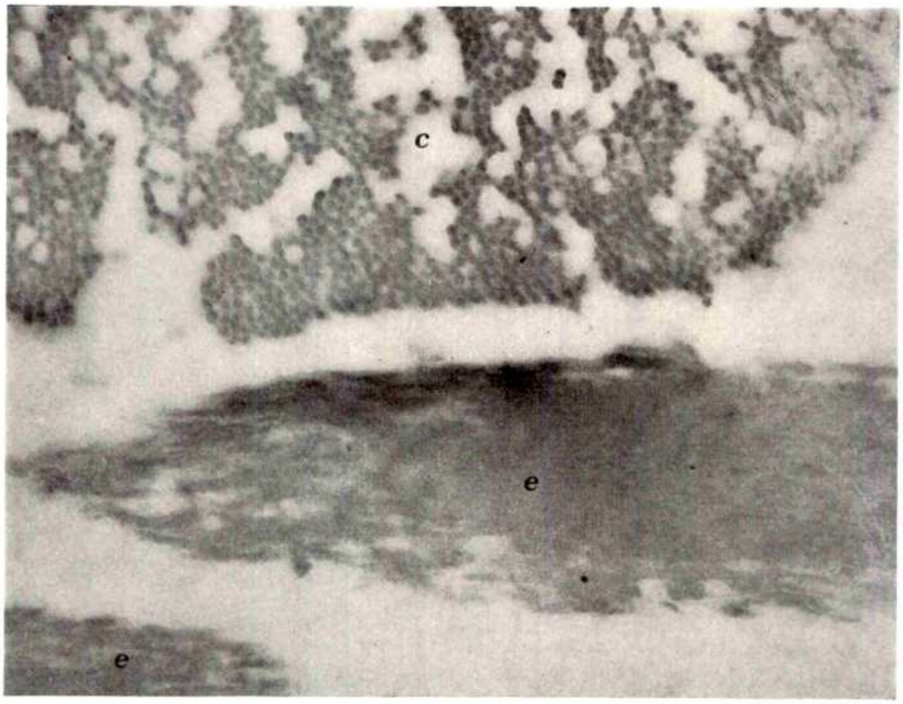

FIG. 9.—The appearance of another collagen bundle (c) and 2 elastic fibres, with similar changes to those described in Fig. 8. (UA—lead citrate × 20,400.)

The " empty spaces " seen around blood vessels, eccrine glands and collagen bundles, in histological sections, bearing in mind also shrinkage artefact and the clinical existence of telangiectasia, suggest a defect in the ground substance. The fact that telangiectasia, after removal of the occlusive dressing from the experimental surface and spraying with CO_2, became larger and more conspicuous suggests that contractile capacity of the blood vessels was preserved and that the amount of ground substance surrounding them was reduced.

It seems that the main change affecting the ground substance is quantitative rather than qualitative.

The appearance of the fibroblasts, as well as the slower but eventual healing of the biopsy wound, suggests incomplete inhibition at the concentration of the corticosteroid used, of their normal function, i.e. the formation of mucopolysaccharides of the ground substance, the production of precursor proteins which, by aggregation, eventually form collagen and elastic fibres. The additional stimulation of the corticosteroid-inhibited fibroblasts by the wound should also be borne in mind.

Mature mast cells were rarely seen. Some authors believe that they are directly implicated in the formation of dermal mucopolysaccharides (Asboe-Hansen, 1950).

In the rabbit the physiological renewal of the dermal mucopolysaccharides is more rapid than that of the collagen and elastic fibres, the half life of hyaluronate being 1·9 days and that of the chrondroitin sulphate fraction 7 days (Schiller *et al.*, 1955). The apparent half life of skin collagen in adult rats is 1,000 days (Thompson and Balou, 1954). The half life of elastic fibres is said to be " long " (Stary, 1963).

In the normal dermis, ground substance is more voluminous than the network of collagen and elastic fibres. It can be presumed that the first changes in our patients occurred in the ground substance, including that between the fibres forming collagen bundles, and that on elastic fibres, as demonstrated electronmicroscopically. A direct effect of corticosteroids on dermal tissue components in addition to that incorporated by its action of the fibroblasts cannot be excluded.

Since the ground substance is the medium through which the transport of nutrients takes place, such a deficit must also influence in various degrees and according to their sensitivity the function of dermal cells which are also directly influenced by corticosteroids.

If the intensity of desired effects of a topical corticosteroid runs parallel and is directly proportional to its inhibitory effect on fibroblasts, more rapid damage of the skin can be expected with the introduction of more potent corticosteroids.

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NAEVUS LIPOMATOSUS CUTANEUS SUPERFICIALIS (HOFFMAN-ZURHELLE)

A. G. FINLEY AND L. A. MUSSO

Summary.—The first example of this rare disorder seen in Australia is presented. The clinical and histological pictures are distinctive, but the precise origin of the condition remains unknown. The various theories relating to aetiology are discussed.

NAEVUS lipomatosus cutaneus superficialis is a very rare disorder. When it was reviewed by Lynch and Goltz in 1958, they could find only 8 examples in the world's literature, and they described a ninth. Since then only a few reports have appeared. It has been regarded as a developmental anomaly, since it has often been noted at birth, and it can be briefly described as the occurrence of ectopic adipose tissue in the corium. It is seen most commonly about the gluteal region, usually appearing as a large plaque of soft yellow papules and nodules.

The similarity between the clinical and histological findings in all the reported cases suggests that this is an entity, and further attention has been drawn to the disorder by the recent reports of focal dermal hypoplasia, which has a similar histological picture.

The report of a typical example of naevus lipomatosus cutaneus superficialis is justified by the extreme rarity of this curious condition.

CASE HISTORY

Mrs A. A., aged 23, presented with a history of lumps on her buttock since the age of 11. These were not growing when she sought advice, although she had noted some recent hair growth on one area. Her general health was good and she had not previously had any skin trouble.

Examination revealed a large plaque over the right gluteal region, with a little hair growing from it (Fig. 1). This plaque was made up of a number of soft papules and nodules which were mostly skin-coloured, although some were pale yellow. The surface was smooth and folded, and some of the individual lesions gave an impression that they could be invaginated slightly on pressure. They were not tender. A provisional diagnosis of a type of connective tissue naevus was made. A biopsy was taken, and subsequently the lesion was partly excised.

The biopsy of a papule showed the same histological features as those seen in the excised specimen, and only the latter will be described. The piece of the excised specimen examined histologically comprised 2 stained sections of total length of 3 cm. Twelve small papules were included. On H. and E. staining, except for the papillary and subpapillary areas, which were essentially free of fat tissue, the dermis of 11 papules showed varying foci of normal-looking fat tissue surrounded by apparently normal connective tissue. The dermis of one papule without any fat tissue was slightly fibrotic. Generally speaking, there were more capillaries in the upper dermis than usual, and the

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ectopic fat tissue was more vascular than the underlying normal fat tissue. On H. and E. staining, the detailed findings of one papule were as follows: there was a slight, diffuse, basket-weave hyperkeratosis and moderate acanthosis, with increased pigmentation of the basal layer (the latter probably normal for this patient). The dermis of this pedunculated papule contained 75% of normal-looking fat tissue (Fig. 2). The papillary and subpapillary connective tissue had more fibroblasts than usual, was slightly fibrotic, and no fat tissue was present (Fig. 3). There was a definite reduction of the elastic tissue in the papillary-subpapillary area (Fig. 4), but it was normal in the connective tissue between the fat foci (Fig. 5).

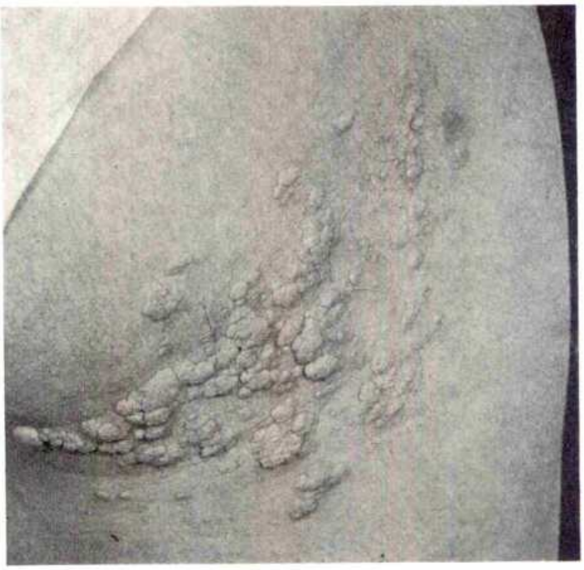


FIG. 1.—The lesion showing the multiple lobules.

On staining with alcian blue pH 2·3 (Fig. 6), there was a moderate increase of alcian blue-positive material between the collagen fibres in the papillary and subpapillary areas at the base of the papule, and to a lesser extent in the same situation over the rest of the papule, while the fat tissue at the base of the papule was slightly positive. The apparently normal H. and E. staining connective tissue between the fat foci was slightly positive between its collagen fibres with alcian blue staining. Further, although elastic tissue occurred without alcian blue-staining material in its immediate vicinity, this may happen and appears to be coincidental.

The stroma of this papule contained 75% of fat tissue which appeared normal except for some increase in vascularity and a partial deposit of what was presumably hyaluronic acid (alcian blue pH 2·3 positive material). It had arisen *in situ* and had not protruded into the dermis from the subcutaneous tissue. The histological findings did not indicate how it was formed.

The reduced elastic tissue, increase of hyaluronic acid and fibroblasts, and slight fibrosis in the papillary-subpapillary area of this papule might have been the result of a previous low grade chronic inflammation or the expression of an abnormality of the connective tissue in this zone. One might suggest that the first possibility is unlikely, since one would not expect an excess of hyaluronic acid, as appeared to be present in

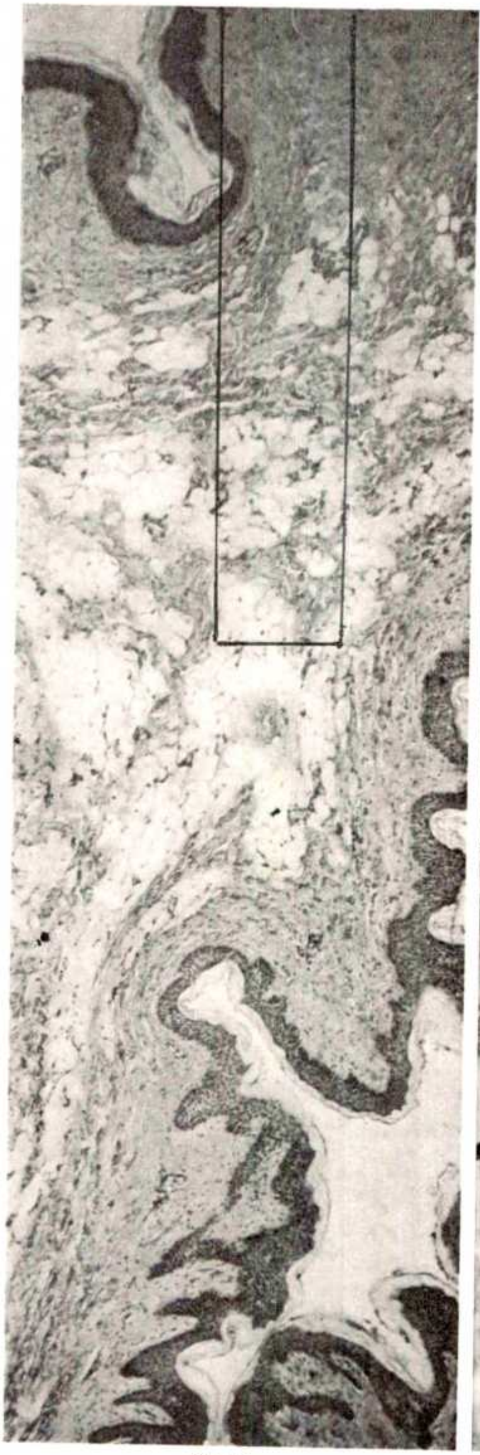


FIG. 2.

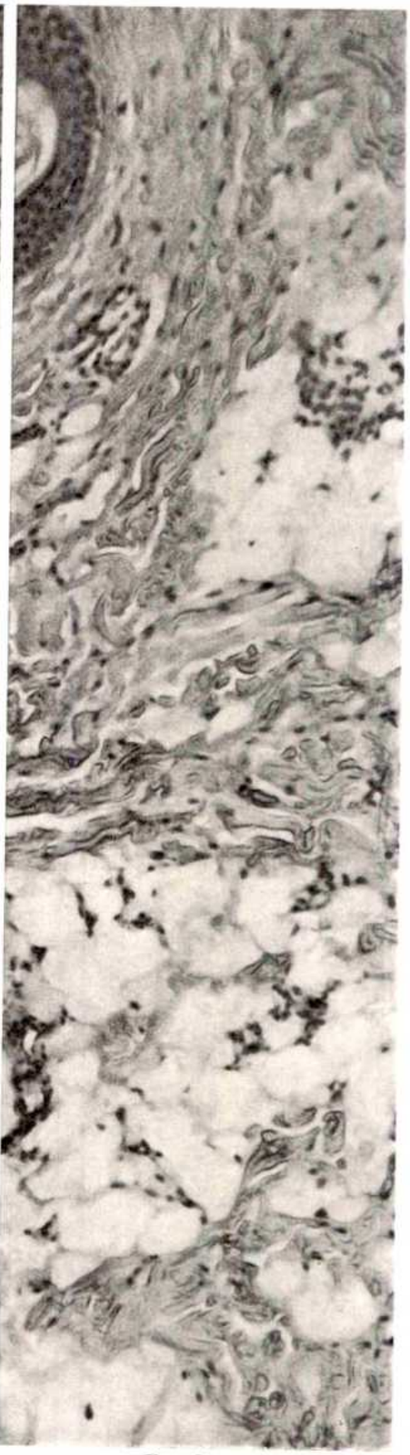
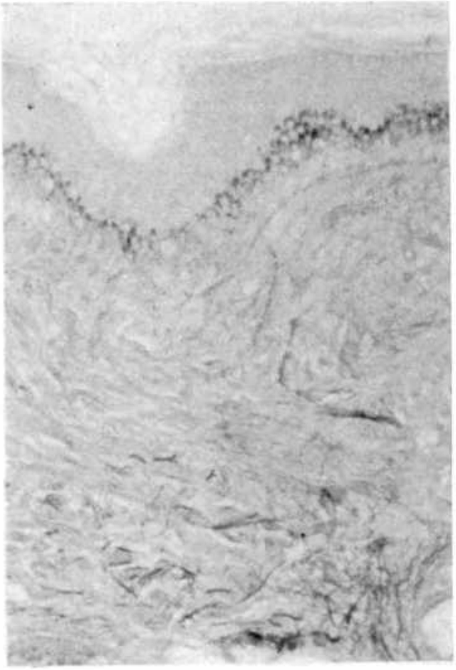


FIG. 3.

FIG. 2.—General view of about half the papule described in the text, showing the amount of fat tissue present. (H. and E. × 50.) FIG. 3.—Approximate enlargement of the inset in Fig. 2. Note, in papillary-subpapillary area, the increase in fibroblasts, the slight fibrosis and the absence of fat tissue. More detail of the fat tissue is visible. (H. and E. × 160.)

FIG. 4.



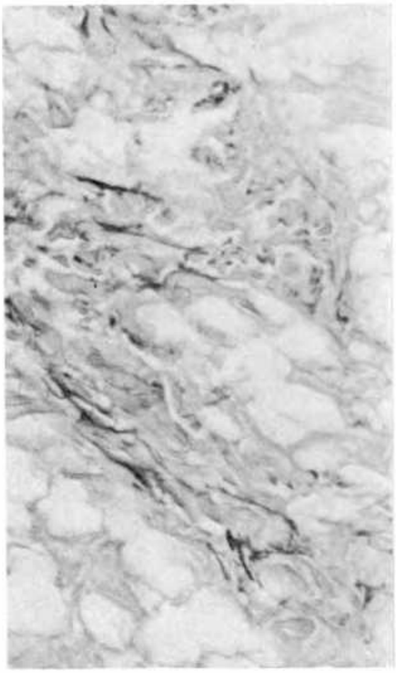


FIG. 5.

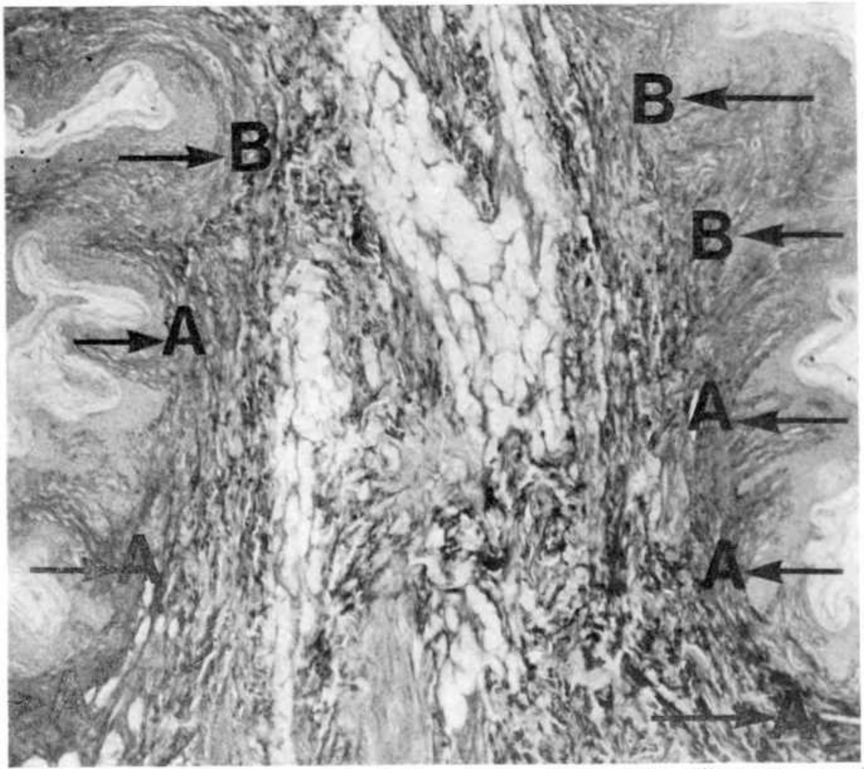


FIG. 6.

FIG. 4.—Top left: shows reduction of elastic tissue in the papillary-subpapillary area near the base of the papule. (Sheridan's modification of Weigert's elastic tissue stain. × 125.) FIG. 5.—Top right: elastic tissue normal in connective tissue between fat foci. (Elastic tissue stain as for Fig. 4. × 125.) FIG. 6.—Bottom: moderate increase of alcian blue pH 2·3 positive material at the base of the papule is arrowed at A, and to a lesser extent at B. (Alcian blue pH 2·3 stain with elastic tissue stain as for Fig. 4. × 60.)

this case, at this stage of such an inflammation. It seems more likely that these effects are the result of some connective tissue abnormality.

Since the fatty tissue abnormality is the most prominent element in this naevoid lesion, it is best classified as naevus lipomatosus cutaneus superficialis with an associated connective tissue malformation.

CLINICAL FEATURES

In most patients at least part of the naevus has been present at birth, but in some it has appeared much later. Holtz (1955) described a patient in whom the lesion appeared at the age of 18. In our patient the first signs became apparent when she was 11.

The predominant site for the lesions is about the pelvic girdle, particularly the gluteal region. Single nodules have been described on the upper thigh (Nikolowski, 1950), the ear (Knoth, 1962) and the scalp (Weitzner, 1968), whilst the larger plaques have also been described in areas such as the abdominal wall (Abel and Dougherty, 1962). These same authors described also a girl aged 6 with lesions over the sacrum, buttocks, deltoids, posterior aspects of the thighs, legs and ankles.

The frequency of these lesions in the pelvic girdle region has aroused some speculation. Nikolowski (1950) wondered if some peculiarity of circulation in this region might be responsible, and he also pointed out that the gluteal region is subjected to persistent pressure during intrauterine life. Holtz (1955) suggested that this region is " lipophilic ", that the fat pad here is usually thick and is frequently a site of predilection for disorders of fat such as lipodystrophies and dystrophia adiposogenitalis. However, lipomata are not common in this area.

It is of interest that the steatopygy of Bushmen and Hottentots manifests itself as prominent fat deposits over the buttocks, and the 1898 edition of Jay Schamberg's " Diseases of the Skin " illustrates a child with " Naevus pilosus et lipomatodes " exhibiting a large lobulated fatty tumour over the right buttock.

Two clinical variants of naevus lipomatosus cutaneus superficialis have been described, the large plaque type and the solitary nodule. In the former the plaques consist of aggregations of flesh-coloured or yellow papules and nodules. They are usually smooth, but may be irregularly folded, or even warty. On palpation the papules and nodules are soft and, as in the case presented here, some can be pressed into the skin. The plaques vary in size and have been known to measure up to 8 × 15 cm. The solitary lesions are similar to the individual papules and nodules seen in the plaque variety.

HISTOPATHOLOGY

Our case conforms to the histopathological features described in other cases. Lynch and Goltz (1958) pointed out that the epidermal changes in general resemble those of epidermal naevi. Hyperkeratosis of basket-weave type, follicular plugging, moderate acanthosis, squaring of rete pegs and increased pigmentation of the basal layer have been described.

The striking changes are seen in the middle and deeper portions of the dermis where there is an extensive deposit of mature adipose tissue. The papillary layer has usually been normal, although we noted some reduction of the elastic tissue, slight fibrosis and increase in fibroblasts.

The adipose tissue shows increased vascularity, with dilated blood vessels coursing through it. In the deeper part of the corium fat surrounds the larger blood vessels. In most cases described, the adipose tissue has not communicated with subcutaneous fat.

Hoffman and Zurhelle (1921) described degenerate collagen tissue and fragmented elastic tissue around the adipose tissue, but these findings have not been consistently reported. Both Kuta (1951) and Thone (1951) saw no collagen degeneration, and elastic fibres were present, although fine and fragmented.

DISCUSSION

Naevus lipomatosus cutaneus superficialis is extremely rare and has features sufficiently distinct to make it an entity. There is, however, very real similarity to certain of the features seen in focal dermal hypoplasia. This syndrome was recently reviewed by Goltz *et al.* (1970) who added 2 new examples to the 41 already reported and indicated that they are aware of 5 other examples which have not been reported. In this disorder a very broad range of developmental anomalies has been described, with involvement of both ectodermal and mesodermal structures. The numerous abnormalities seen in the syndrome were summarized by Goltz *et al.* under 6 headings: general features, cutaneous abnormalities, skeletal defects, ocular abnormalities, oral and dental changes, and soft tissue defects. The name of the syndrome derives from one of its most spectacular features, the areas of thin, undeveloped skin through which the subcutaneous fat herniates. Howell (1965), in reporting 2 patients he described under the title " angiolipomatosis *vs.* focal dermal hypoplasia ", drew attention to the fact that there was histological similarity between focal dermal hypoplasia and naevus lipomatosus. In both disorders adipose tissue is seen in the dermis, often in relation to dermal blood vessels. They can nevertheless be distinguished by virtue of the fact that there is an extreme degree of attenuation of collagen in focal dermal hypoplasia.

There is of course a vast clinical difference between the two, as no associated abnormalities have ever been described with naevus lipomatosus superficialis.

The origin of this unusual disorder remains obscure, and the technical difficulties associated with the study of adipose tissue have not helped to bring a solution nearer. Adipose tissue in general should be regarded as an organ, a specific tissue which is not merely a modified connective tissue (Cairns, 1968). It consists of aggregations of fat cells in a collagenous framework which are found throughout connective tissue, but distribution is not uniform. In certain sites, such as the buttocks, thighs, shoulders and abdominal wall, it is present in increased quantities.

There is still not complete agreement as to the development of adipose tissue. Ambiguity surrounds the cell of origin. It may be a perivascular reticular cell or a stem cell apparently indistinguishable from a typical fibroblast; or it may be that fat cells are derived from endothelium, or that both fat cells and endothelium arise from a common precursor. While fat cells may arise from endothelium, by the time they are differentiated sufficiently to permit recognition as fat cells, they are already perivascular in position.

Wasserman (1926) postulated the development of adipose tissue from primitive organs in embryonic connective tissue, ovoid bodies with an associated network of capillaries. Simon (1965) does not believe the term " primitive fat organ " is justified, but he and Wasserman agree that primitive adipose cells originate in perivascular cells, and it appears that the differentiation of fat cells is intimately related to blood vessels.

Various theories have been put forward to explain the pathogenesis of naevus lipomatosus cutaneus superficialis. These include the following:

(1) Hoffman and Zurhelle (1921) considered the deposition of fat to be secondary to some degenerative change in connective tissue, basing this on the presence of degenerate collagen and fragmented elastic tissue. Nikolowski (1950) agreed on the basis of seeing similar degenerative changes, but their findings have not been seen uniformly in later examples of the disorder. Although we saw changes in the papillary and subpapillary zone suggestive of connective tissue abnormality, we do not feel there is anything to suggest it was a primary change.

(2) Holtz (1955) advanced the theory that the primary disturbance is the growth of adipose tissue from blood vessels, and that the degenerative changes in the connective tissue are secondary. His belief was based on the observation that there were not only large mature fat cells but also immature cells, as are seen in embryonic adipose tissue. His conclusion that primitive " preadipose " tissue grows from blood vessels, invades the connective tissue and destroys it, conforms with accepted beliefs about the normal embryonic development of adipose tissue. Lynch and Goltz (1958) supported this view.

(3) Metaplasia of collagen tissue, with its transformation into fat, has been suggested by way of explanation, but there does not appear to be enough change in the adjacent corium to support this belief. Some changes in connective tissue have been noted, but they have not been of sufficient magnitude to provide evidence to substantiate this theory. However, it appears that the development of adipose tissue by metaplasia of connective tissue may occur, and the occurrence of fat in the stroma of glands, often the parotids of old people, has been put forward as evidence of this.

(4) Focal heterotopic development of adipose tissue in the corium was proposed as a likely explanation by Robinson and Ellis (1937). This suggests that displacement of subcutaneous adipose tissue into the dermis takes place during foetal development. The fact that the dermal fat deposits are not contiguous with the subcutaneous fat does not entirely negate this possibility.

In the light of present knowledge there appears to be no way to resolve this question. The findings in the case described do not shed any light on the aetiology.

We wish to thank Dr E. Hirst, Director of Morbid Anatomy, and Mr R. A. J. Money of the Department of Clinical Photography, Sydney Hospital for the production of the photomicrographs.

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GENERALIZED *TRICHOPHYTON MENTAGROPHYTES* RINGWORM IN CAPUCHIN MONKEYS (*CEBUS NIGRIVITATUS*)

B. G. BAGNALL AND W. GRÜNBERG

Summary.—A chronic and extensive *Trichophyton mentagrophytes* infection is reported in 3 Capuchin monkeys (*Cebus nigrivitatus*). The animals were severely debilitated from internal parasites, pneumonia and enteritis, which led to their deaths within 2 months of purchase by a zoo. Clinically there was widespread hair loss, with skin scaling and crusting. The histological picture was one of marked hyperkeratosis and peri-folliculitis. The disease was not transmitted to 5 other healthy monkeys housed in the same enclosure.

IT is commonly believed by veterinary surgeons that animals which suffer from a chronic and extensive ringworm infection are often debilitated from secondary disease factors, including malnutrition, parasitism and stress from overcrowded housing. Ringworm in primates is not uncommon, but the infection is usually by *Microsporum* species, and an association with intercurrent disease has rarely been described.

CASE REPORTS

Three adult Capuchin monkeys (*Cebus nigrivitatus*) were purchased from an animal dealer by a zoo in Vienna. The previous history of the animals is unknown. On arrival at the zoo their obviously poor condition and " sparse hair coat " were put down to inadequate feeding by the the previous owner. They were housed in an enclosure which contained 5 other adult Capuchin monkeys (1 *Cebus apella*, 2 *Cebus albifrons* and 2 *Cebus capucinus*). A week later one of the newly arrived monkeys died and was brought for post mortem examination. Among other diseases, the animal was found to be suffering from extensive ringworm. A close look at the remaining monkeys revealed similar lesions only in the other 2 new arrivals. No treatment was given, and within 2 months both these monkeys had also died after becoming ill with diarrhoea and respiratory distress. The other 5 monkeys in the same enclosure remained healthy and free from any signs of skin disease.

Post mortem findings. Monkey No. 1.—female, emaciated. Generalized hair loss; skin scaling and crusting. Catarrhal bronchitis; pneumonia; pleurisy; lungworm infestation. Chronic valvular endocarditis. Splenic enlargement. Chronic catarrhal enteritis; parasitic nodules in bowel wall. *Monkey No.* 2.—female, well nourished. Extensive hair loss; skin scaling and crusting on back, neck and arms. Focal pneumonia; purulent bronchitis; lungworm infestation. Hepatic congestion. Parasitic nodules in bowel wall. Splenic enlargement. *Monkey No.* 3.—male, very much emaciated. Generalized hair loss; thick scaling and crusting of skin. Anaemia. Lungworm infestation. Hepatic enlargement; jaundice. Catarrhal enteritis.

Parasitology.—Unidentified filarial lungworms; *Molineus tolurosus* and *Oesophagostomum* sp. in gut.

Mycology.—Samples of skin and hair confirmed a heavy dermatophyte infection. KOH preparations showed most hairs to be surrounded by a sheath of medium-size

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fungal arthrospores. Cultures on Sabouraud's agar resulted in the growth of *Trichophyton mentagrophytes* (Robin) Blanchard, 1896.

Skin lesions.—These were similar in all 3 monkeys. There was extensive hair loss, with bran-like scaling and the formation of thick crusts, particularly down the back (Fig. 1). Where small tufts of hair remained, the shafts could easily be pulled out with a gentle tug of the fingers. In some areas a pyoderma had developed, with discharge of pus between the crusts.

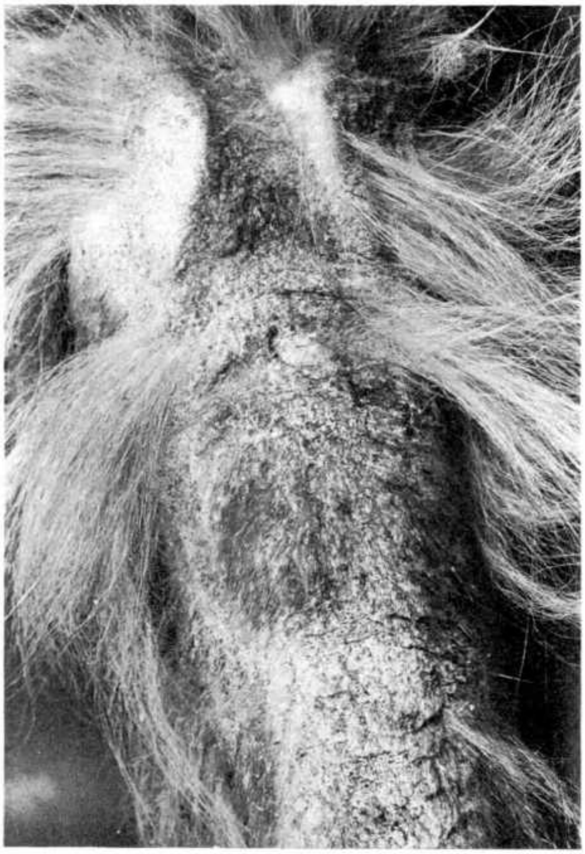

FIG. 1.—The back of Monkey No. 3, showing the extent of the hair loss, together with bran-like scaling and the formation of thick crusts on the skin. Prominent scapulae indicate the animal's emaciated condition.

Skin histology.—There was marked hyperkeratosis, with loose sheets of flaky keratin. The upper portions of the hair follicles were also hyperkeratotic, resulting in an almost papillomatous appearance from dilatation of the infundibula with layers of keratin (Fig. 2). Even H. and E. staining clearly showed masses of fungal arthrospores surrounding the hairs. There was very little epidermal inflammatory reaction. The striking histological finding was that of peri-folliculitis. In most sections many follicles could be seen surrounded by an inflammatory infiltrate of polymorphs and lymphocytes. Intrafollicular abscesses were also common, and follicles were so much damaged as to lead to their rupture and the formation of dermal abscesses containing hair fragments (Fig. 3).

The associated inflammatory infiltrate contained only occasional foreign-body giant cells. PAS staining showed large numbers of fungal elements in these dermal abscesses and granulomata. All the free hair fragments were covered with arthrospores and hyphae. More puzzling, however, was the frequent finding of apparently active-growing hyphae amongst the inflammatory cells in the dermis without any definite hair being present (Fig. 4). A careful search always revealed many macrophages engorged with melanin granules in the vicinity, indicating the possible digestion of former hair fragments.

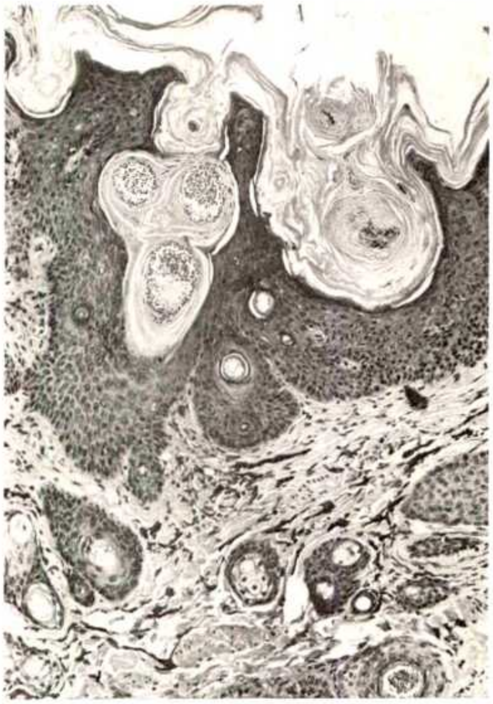

FIG 2.—The upper portions of the hair follicles are widely dilated with layers of keratin to give an almost papillomatous appearance. Even H. and E. stain shows masses of fungal arthrospores surrounding the hairs, but PAS staining was required to show the network of hyphae in the hyperkeratotic stratum corneum. The infection provokes little epidermal inflammation, but is accompanied by acanthosis and pigmentary incontinence into the upper dermis. (H. and E. × 150.)

DISCUSSION

Trichophyton mentagrophytes has rarely been reported as a cause of monkey ringworm (Dawson, 1968). A similar species was isolated by Emmons (1940) from a Rhesus monkey, but this has now been shown to be *T. simii* (Stockdale *et al.*, 1965). *T. mentagrophytes* has also been isolated from a normal monkey, and it was thought at the time that these animals may act as " carriers " for this dermatophyte (Refai and Bisping, 1964). Considering the frequent association of rats and mice with monkey enclosures in zoos, it is surprising that *T. mentagrophytes* ringworm is not more common in these primates. The effect of intercurrent disease has also seldom been reported, although Koch and Jänisch (1964) found liver abscesses in a Rhesus monkey with an extensive *Microsporum gypseum* infection.

The 3 monkeys reported here were all heavily parasitized with worms and died within 2 months of purchase after suffering from pneumonia and enteritis. The relationship of their debilitated condition and ill-health to the extent and chronicity of their ringworm infection can only be speculated. Sellers *et al.* (1956) noted that severe and extensive *T. verrucosum* ringworm in cattle occurred mostly in undernourished animals. The monkeys obviously had the skin disease before purchase, and thus the origin of the infection is unknown. It is interesting to note that none of the other 5 healthy monkeys in the same enclosure developed any evidence of ringworm, despite the fact that the 3 infected animals were virtually covered with lesions and in close contact with them.

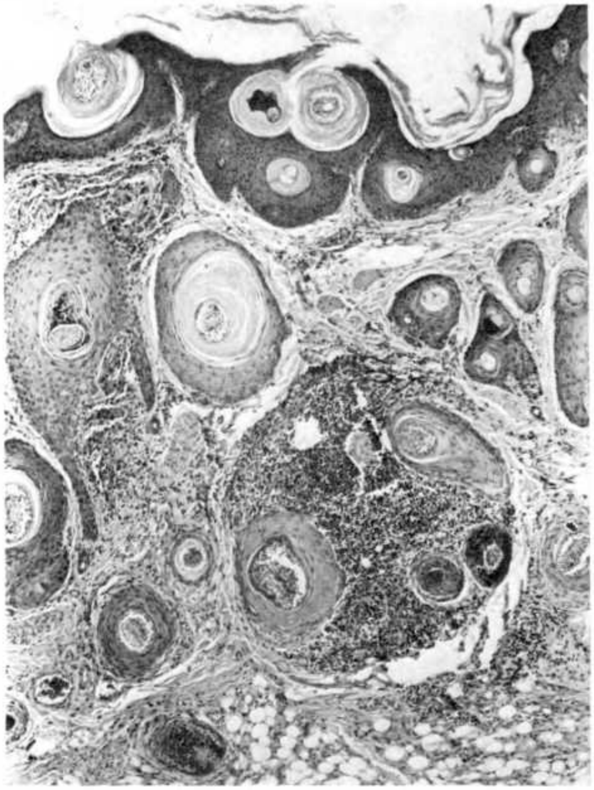

FIG. 3.—A dermal abscess has resulted from rupture of hair follicles which were damaged by intra- and peri-folliculitis. A free hair fragment is visible in its centre, but foreign-body giant cells are rare. (H. and E. × 150.)

The histological picture was one of marked hyperkeratosis, with intra- and peri-folliculitis. Baker *et al.* (1971) found similar changes in a Rhesus monkey with a severe *M. canis* infection, but noted also the presence of numerous giant cells in the dermis where hair follicles had ruptured. The finding of actively-growing fungal hyphae in the middle of inflammatory foci in the dermis suggests that enough keratin remains after digestion of hair fragments to allow further growth of the dermatophyte. These dermal lesions are similar to the Majocchi granuloma of man.

No treatment was carried out, although it is well known that griseofulvin therapy is rapidly effective, even in advanced cases. The need for an early diagnosis of ringworm in monkeys is emphasized by the fact that the disease in this species is often accompanied by pruritus and that a general scaling of the skin with hair loss is often found in the absence of more typical ring-shaped lesions (Ruch, 1959).

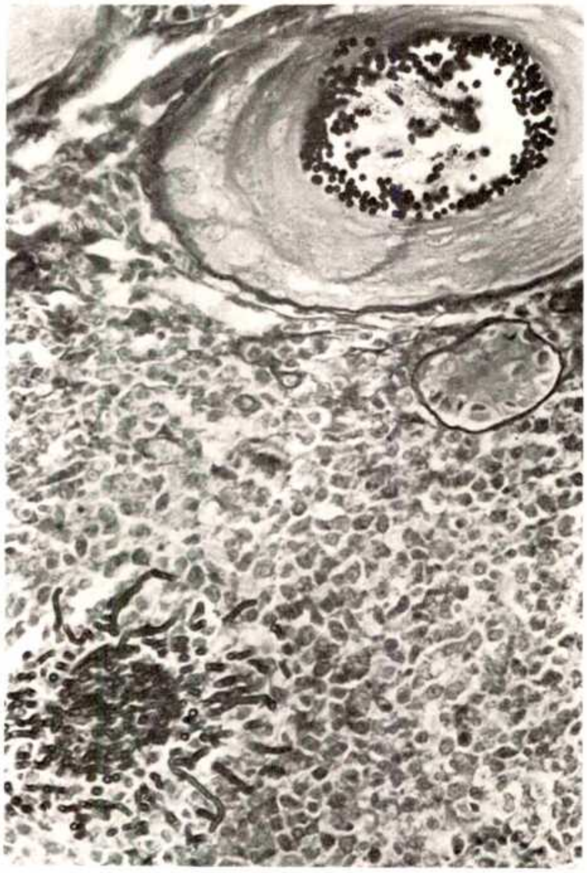

FIG. 4.—High power of a dermal abscess. The hair follicle above is infected by the dermatophyte, and masses of arthrospores are visible in and around the hair shaft. As yet the follicular wall is intact. Lower left shows actively-growing hyphae amongst inflammatory cells. A follicle has been totally destroyed here, and the fragment of hair probably digested, but leaving enough keratin to allow further growth of the fungus. (PAS, × 400.)

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CYCLIC 3′,5′ ADENOSINE MONOPHOSPHATE-DEPENDENT PROTEIN KINASE OF SKIN

I. MEASUREMENT AND PROPERTIES

P. D. MIER AND J. VAN DEN HURK

Summary.—The presence of cAMP-dependent protein kinase activity has been confirmed in skin homogenates. Half-maximal stimulation was observed at about 5×10^{-8} mol/1 cAMP, which is very similar to figures reported for protein kinase preparations from other tissues. The apparent K_m for ATP, however, was lower than values previously reported, and was unaltered by the presence of cAMP.

Cyclic 3′,5′ adenosine monophosphate (cAMP) is now firmly established as the intracellular mediator for many different hormones. Nevertheless, the mechanism by which this substance exerts such a profound metabolic influence on diverse metabolic pathways within the cell has only recently been elucidated.

The first insight into the problem was provided by Walsh *et al.* (1968), who isolated a protein kinase from rabbit muscle which catalysed the phosphorylation (and consequent activation) of phosphorylase kinase. Their preparation was stimulated by cAMP in concentrations of the order of 10^{-7} mol/l. In the following year Kuo and Greengard (1969) demonstrated that cAMP-dependent protein kinase activity was present in numerous mammalian tissues, and in species representative of many different phyla. Soderling *et al.* (1970) showed that the same enzyme was responsible for the phosphorylation (with, in this case, the inactivation) of glycogen synthetase. In addition, purified preparations from a number of sources have now been described in detail; most of these are quite similar in their properties. Thus there is now little doubt that cAMP-dependent protein kinase is an essential component in the cAMP-mediated response.

An investigation of the cAMP-dependent protein kinase activity of skin was therefore undertaken in these laboratories; the results are presented below. During the course of this work a similar report has been published elsewhere (Kumar *et al.*, 1971).

MATERIALS

Reagents were in general of analytic grade. Adenosine triphosphate (ATP) and histone were purchased from the Sigma Chemical Co., U.S.A.; the latter was the lyophilized preparation from calf thymus (Sigma type II A). ATP-γ-^{32}P was obtained from the Radiochemical Centre (Amersham, England) at a specific activity of about 2,000 mCi/mmol.

All specimens of skin were from the backs or flanks of albino mice; the animals were killed by dislocation of the neck, shaved; and the skin removed. After dissecting off

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the subcutaneous muscle, the weighed specimen was homogenized in the appropriate medium, using a " Micro-wet Grinder " (Townson and Mercer, England) which had been modified by the inclusion of an ice-jacket. ^{32}P activity was measured using an end-window G-M counter.

EXPERIMENTAL

Assay procedure

The final composition of the reaction mixture was exactly as described by Soderling *et al.* (1970) for the phosphorylation of histone.

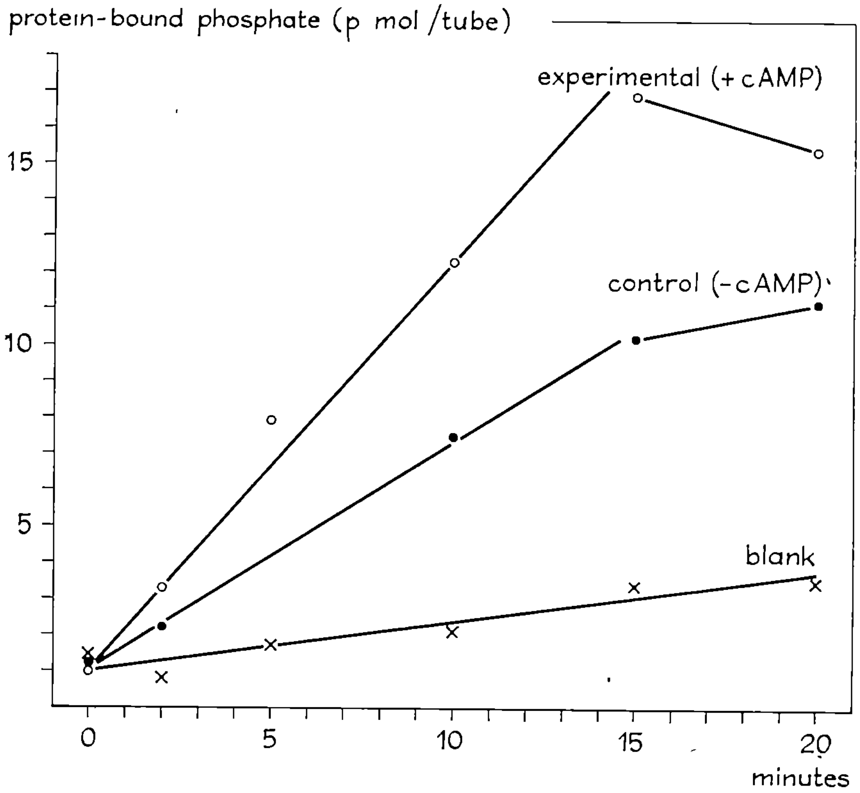

FIG. 1.—Time-course of the phosphorylation of histone. Conditions are as described in in the text.

A " stock buffer " was prepared containing 125 mmol/l 2-[N-morpholino]-ethane sulphonic acid, 2·5 mmol/l K_2HPO_4, 50 mmol/l NaF, 0·75 mmol/l EGTA, 0·5 mmol/l EDTA, 5 mmol/l theophylline, and 25 mmol/l magnesium acetate. The pH was adjusted to 6·7. Histone (60 mg/ml) was dissolved in stock buffer (" buffered histone "); this was kept at 0°C and prepared freshly at weekly intervals. A solution of ATP was prepared at 4 mmol/l (pH 6·7), containing approximately 50 μCi ^{32}P/ml, and a solution of cAMP at 20 μmol/l. Skin was homogenized in a dilution of 400 ml/l of the stock buffer in water, and the homogenate centrifuged to yield a clear supernatant.

A mixture consisting of 0·1 ml buffered histone, 0·05 ml ATP, 0·05 ml cAMP, and 0·05 ml water was made in a centrifuge tube at 0°C. The reaction was initiated by the addition of 0·25 ml skin extract, and the tube transferred to 30°C. A control tube, in which the cAMP was replaced by water, and a blank (in which the skin extract was replaced by dilute buffer) were invariably included. After an appropriate interval (usually 10 min), the reaction was terminated by the addition of 0·5 ml 10% trichloroacetic acid, and the mixture centrifuged. Soluble phosphate was removed by redissolving the precipitate in 1·0 ml of 0·1 mol/l NaOH, and re-precipitating with 1·0 ml trichloroacetic acid. After 3 such cycles, the final precipitate was re-dissolved in 0·4 ml of 0·2 mol/l NaOH, and an aliquot transferred to a planchette for measurement of ^{32}P activity. Protein-bound phosphate was calculated as 10^{-12} mol P; enzymatic activity was expressed as 10^{-9} mol P bound per min per g wet weight skin.

RESULTS

The values obtained for cAMP-dependent protein kinase activity in the skin of 5 individual animals are shown in Table I; all figures have been connected by subtraction of the corresponding blank. It is seen that the activity in the presence of added cAMP is approximately double that of the control.

Time Curves

Preliminary experiments were carried out to determine the time during which the reaction rate remained constant. Incubation mixtures with and without added cAMP, and a blank (omitting the skin extract) were set up. The total

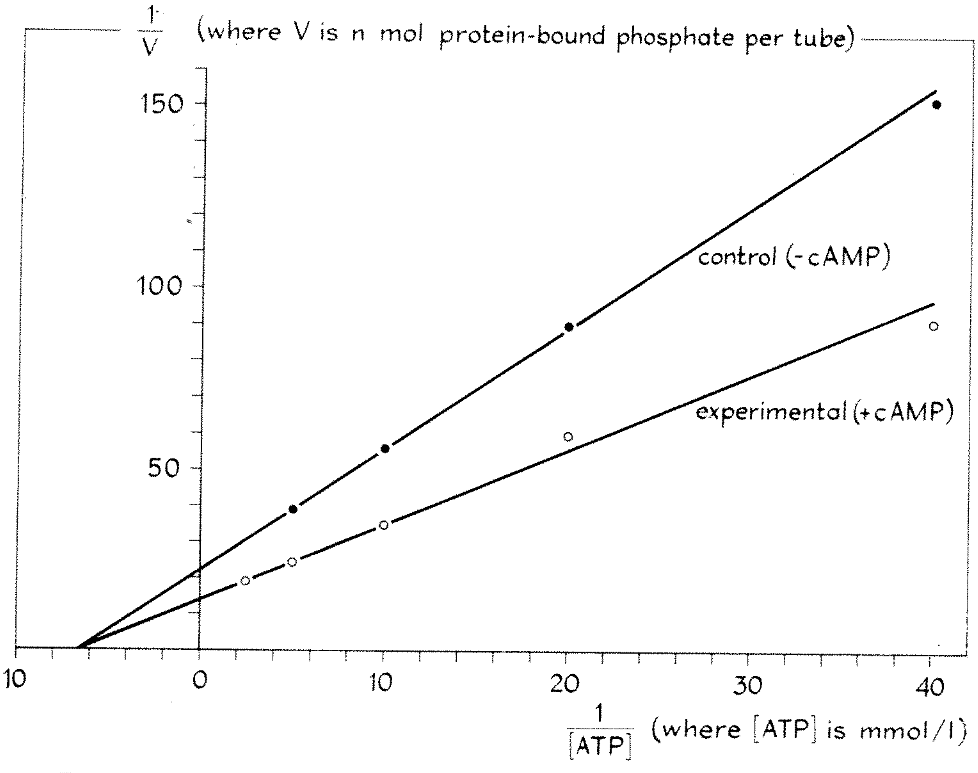

FIG. 2.—Lineweaver-Burk plots, showing the variation in reaction velocity at different concentrations of ATP.

TABLE I.—*Values of cAMP-dependent Protein Kinase Activity in Mouse Skin. All Figures Have Been Corrected by Subtraction of the Corresponding Blank*

	Activity (10^{-9} mol/min./g wet wt.)	
Specimen no.	Control (−cAMP)	Experimental (+cAMP)
1	2·4	4·8
2	2·6	4·4
3	1·2	2·8
4	2·7	4·3
5	1·7	2·9
Mean	2·1	3·8

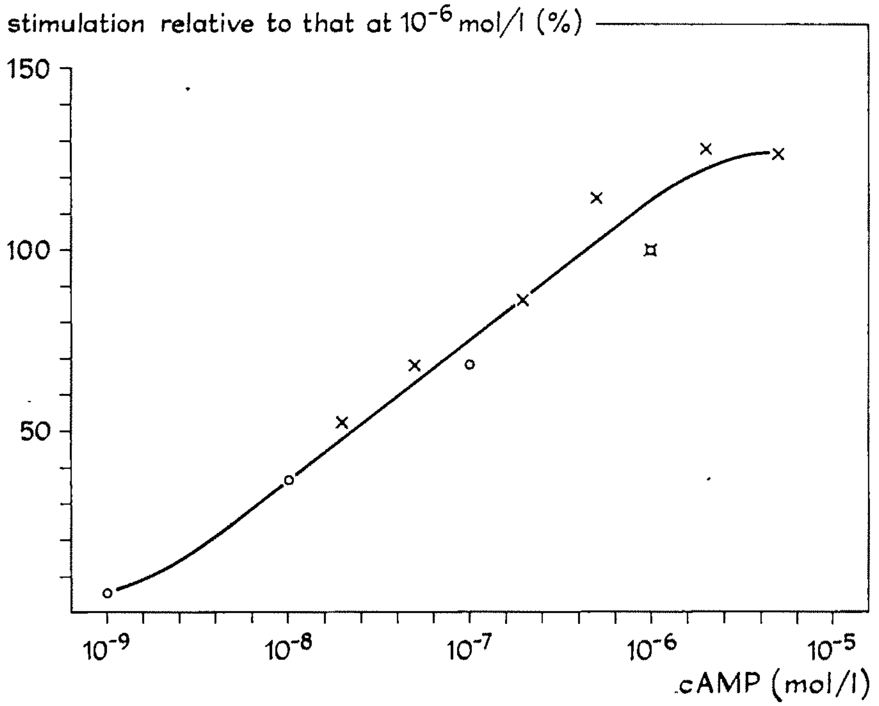

FIG. 3.—Stimulation of cutaneous protein kinase activity by various concentrations of cAMP expressed as a percentage of that measured at 10^{-6} mol/l cAMP

incubation volumes were 1·5 ml in each case; 0·2 ml aliquots were pipetted into 0·5 ml portions of trichloroacetic acid at intervals up to 20 min, and the assays completed as before.

Typical curves, using a skin extract of 20 mg/ml, are shown in Fig. 1. At higher concentrations of skin (such as 50 mg/ml) the initial reaction rate was correspondingly greater, but the reaction remained linear only for a very short period.

Effect of Varying Concentration of ATP

Dilutions of the ATP solution were prepared down to 250 μmol/l, and assays conducted using a skin extract of 20 mg/ml and an incubation period of 5 min. A separate blank was included at each individual ATP concentration.

The results are illustrated in Fig. 2 as double reciprocal (Lineweaver-Burk) plots; all figures have been corrected by subtraction of the blank value. Extrapolation yields an apparent K_m of about $1{\cdot}5 \times 10^{-4}$ mol/l for ATP; this figure is independent of the presence or absence of cAMP.

Effect of Varying the Concentration of cAMP

Dilutions of cAMP were prepared down to 10^{-8} mol/l, and assays conducted using a skin extract of 20 mg/ml and an incubation period of 10 min.

The results of 2 individual experiments are illustrated in Fig. 3. Again, all figures have been corrected for the blank; stimulation is expressed as the percentage increase in activity over the corresponding control assay.

DISCUSSION

The presence of cAMP-dependent protein kinase in skin has been confirmed. The sensitivity of the isotopic assay technique, in our hands, proved adequate; however, the low activity of skin, coupled with a rather high blank, tended to reduce the overall reproducibility. It is likely that the use of a lysine-rich or arginine-rich histone preparation would result in a marked improvement; but, for routine use, these materials are prohibitively expensive.

The sensitivity of cutaneous protein kinase to cAMP is very similar to values quoted for other tissues; half-maximal stimulation occurs at about 5×10^{-8} mol/l cAMP, and maximal stimulation is reached at about 10^{-6} mol/l. Our curve for mouse skin (Fig. 3) is in good agreement with that found by Kumar *et al.* (1971) for human skin. The magnitude of the stimulatory effect, on the other hand, seems to be very much dependent on the source of the enzyme. Our finding of a 2-fold stimulation is of the same order as that reported by Kumar *et al.* (1971); however, Kuo and Greengard (1969) have examined a range of tissues from several different mammalian species, and report stimulations varying from less than 2-fold to more than 10-fold. Since extensive purification of the preparation generally increases the apparent stimulatory effect of cAMP, it is likely that non-specific protein kinases are present to a significant extent in most tissues.

Extrapolation of the Lineweaver-Burk plot for ATP yields an apparent K_m of $1{\cdot}5 \times 10^{-4}$ mol/l, regardless of whether cAMP is present or absent. Miyamoto *et al.* (1969) reported an apparent K_m for the bovine brain enzyme of $2{\cdot}2 \times 10^{-4}$ mol/l in the absence of cAMP, falling to $1{\cdot}3 \times 10^{-5}$ mol/l at maximal stimulatory levels. Reimann *et al.* (1970), however, using rabbit skeletal muscle, reported a K_m of about $1{\cdot}5 \times 10^{-5}$ mol/l, which was unaffected by cAMP. Again, it is not clear to what extent these figures represent real tissue and species differences; the higher values may (at least in part) relate to non-specific protein kinase activity.

Because of the key position of this enzyme in the cAMP-mediated response, it is an exciting possibility that small differences in its kinetic properties might form a major determinant of the metabolic pattern peculiar to any particular cell type. Thus it is clearly of importance that more precise characterization of preparations from various sources be carried out; this work is currently in progress in a number of laboratories.

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CYCLIC 3′,5′ ADENOSINE MONOPHOSPHATE-DEPENDENT PROTEIN KINASE OF SKIN

II. LEVELS IN ATOPIC DERMATITIS AND PSORIASIS

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E. P. M. J. HOLLMAN, J. E. PORTERS AND M. B. M. WEEMERS

Summary.—cAMP-dependent protein kinase activity has been measured in skin biopsies from atopic patients, psoriatic patients, and from healthy volunteers. No significant differences were observed either in the presence or absence of cAMP.

IN 1968 Szentivanyi proposed the " beta-adrenergic " theory of atopy, in which he postulated a functional deficiency of the enzyme adenyl cyclase. Mier and Urselmann (1970) were unable to demonstrate any abnormality in this enzyme in the skin of patients with atopic dermatitis. In an extension of this investigation, the degradative enzyme, cAMP phosphodiesterase, was examined and also found to be normal (Holla *et al.*, 1972). We have now turned our attention to the final enzyme of this group, cAMP-dependent protein kinase; clearly a block at this point in the pathway might produce effects very similar to those resulting from a hypothetical adenyl cyclase deficiency.

More recently, an abnormality in the cAMP-mediated system has been implicated in a very different skin disease. Voorhees and Duell (1971) have pointed out that a reduced beta-adrenergic response would lead to an increase in mitotic rate (via loss of inhibition by the chalone–adrenaline complex), and would also shift the glycogen balance in the direction of synthesis (increased activation of glycogen synthetase and reduced activation of phosphorylase). These workers have therefore postulated such an abnormality in the skin of the psoriatic.

In this communication we present direct measurements of the protein kinase activity in the skin of atopic patients, psoriatic patients and healthy controls.

MATERIALS AND METHODS

All reagents were as described in the previous paper (Mier and v. d. Hurk, 1972). Patients were selected who had a clear-cut diagnosis of atopic dermatitis; these had chronic flexural eczematous lesions, and showed a positive reaction to intradermal allergen tests. The psoriatic patients were mainly attending the in-patient department. As far as possible, biopsies were taken immediately after admission and before therapy had begun. The control biopsies were from healthy volunteers.

Biopsy was performed with a 4 mm punch after freezing the skin with ethyl chloride spray. The whole specimen (6–21 mg fresh weight) was homogenized in 1 ml buffer and centrifuged. Assay of protein kinase activity was carried out on 0·25 ml portions of the supernatant, exactly as described in the previous paper (Mier and v. d. Hurk, 1972). A blank was included with each measurement.

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TABLE I.—*Protein Kinase Activity in the Skin of Patients with Atopic Dermatitis, Patients with Psoriasis, and Healthy Controls. All Values are* 10^{-9} *mol/l Phosphate Bound to Histone/min/g Fresh Weight of Skin*

	Atopic				Psoriatic					
	Lesion		" Uninvolved "		Lesion		" Uninvolved "		Controls	
Specimen no.	−cAMP	+cAMP	−cAMP	+cAMP	−cAMP	+cAMP	−cAMP	+cAMP	−cAMP	+cAMP
1	1·17	2·08	0·82	0·91	1·89	3·56	0·31	1·17	0·63	1·39
2	3·00	3·54			2·98	4·34	0·96	2·06	0·55	0·59
3	1·98	3·28			0·74	1·02	1·20	1·31	0·67	1·12
4	1·15	1·67			3·13	5·67	0·15	0·79	1·81	1·98
5									0·29	1·03
6									0·36	1·51
7									0·13	0·81
Mean	1·80	2·63	0·82	0·91	2·19	3·65	0·66	1·33	0·63	1·20

RESULTS

The protein kinase activity in 5 biopsies from atopic patients, 8 biopsies from psoriatic patients and 7 control biopsies is summarized in Table I.

DISCUSSION

Clearly neither the 4 specimens of clinically " uninvolved " psoriatic skin nor the single biopsy of " uninvolved " atopic skin differ significantly from the control values. In the case of the lesions it is necessary to make allowance for the increased cellularity of the tissue. This has been done by expressing the activity in terms of the average DNA content (Mier and McCabe, 1963); the values obtained are shown in Table II. Again we find no evidence for any reduction in either the total activity or the degree of cAMP stimulation in either of these diseases.

TABLE II.—*Protein Kinase Activity Expressed in Terms of the DNA Content of the Skin. None of the Differences are of Statistical Significance*

	Protein kinase activity (10^{-3} mol/min/mol DNA phosphorus)	
Tissue	−cAMP	+cAMP
Atopic lesion	0·28	0·41
Psoriatic lesion	0·40	0·67
Controls	0·22	0·42

With regard to psoriasis, we can say only that we are unable to demonstrate an abnormality in the cAMP-dependent protein kinase; clearly further work is required before Voorhees's hypothesis can be substantiated or rejected. With regard to atopic dermatitis, however, we have now examined all 3 of the enzymes which are directly concerned with the metabolism of cAMP. Our consistently negative findings clearly strengthen the view expressed by Cotton and v. d. Hurk (1972) that the " inbalance " shown by skin and lung of the atopic may well be an overstimulation by substances such as acetylcholine or histamine, rather than an unresponsiveness to beta-adrenergic stimulation.

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EFFECTS OF CYTOCHALASIN B ON EPIDERMAL CELLS *IN VITRO*

HILARY CONSTABLE, C. N. D. CRUICKSHANK AND PATRICIA R. MANN

Summary.—Effects of cytochalasin B have been studied on organ cultures and dispersed cell cultures of adult guinea-pig ear skin, using electron microscopy and time-lapse cinephotomicrography. Cytochalasin B failed to inhibit epiboly at 1 μg/ml, caused slight inhibition at 2 μg/ml and total inhibition at 5 μg/ml. These concentrations did not appear to affect ruffling of membranes or internal cell movements. Some effect was demonstrated at 20 μg/ml. These concentrations, however, caused a general toxic effect on the cells. Ultrastructurally, there was possibly some reduction in the microfilament system, but this was far from dramatic. Abnormalities caused were increased numbers of lysosomes and dilation of the endoplasmic reticulum.

THE cytochalasins are a group of substances isolated from several species of fungi and which have remarkable biological effects on living cells. Cytochalasin B, which is derived from *Helminthosporum dematoideum*, has been the most widely used cytochalasin. The effects include interference with division of the cytoplasm, inhibition of cell movement in macrophages (Allison *et al.*, 1971) and fibroblasts, and extrusion of the nucleus in the latter (Carter, 1967). The cytochalasins also interfere with morphogenesis (Wessells *et al.*, 1971) and embryogenesis (Linville and Shepard, 1972). There is some evidence that these effects may be caused by interference with the microfilament system of the cells. This aspect is reviewed by Wessells *et al.* (1971); Carter (1972), however, urges caution in relation to this concept, as there is yet no direct evidence for it.

All systems so far studied have been *in vitro*, and in general it is not easy to find systems *in vivo* in which to test the effects of the cytochalasins. Since, however, under conditions of organ culture, cell movement is essential to the process of epiboly, it seemed of interest to examine the effects of cytochalasin B on this phenomenon. A study was also made of its effects on cultures of epidermal cells in monolayer by time lapse cinephotomicrography. The ultrastructure of normal and treated cells was also examined.

MATERIALS AND METHODS

The method of organ culture described by Medawar (1948) and later modified by Cruickshank (1951) was used. Essentially, thin slices of guinea-pig ear skin are floated on culture medium and incubated at 37°C. In this instance the medium consisted of MEM 80%, calf serum 20%, buffered with H.E.P.E.S. buffer (Fisk and Pathak, 1969) and containing penicillin and streptomycin at 100 μg/ml. Cytochalasin B was dissolved in dimethyl sulphoxide (DMSO) at a concentration of 1% w/v. Three sets of cultures in triplicate were incubated for 3 days. The experimental cultures had cytochalasin B

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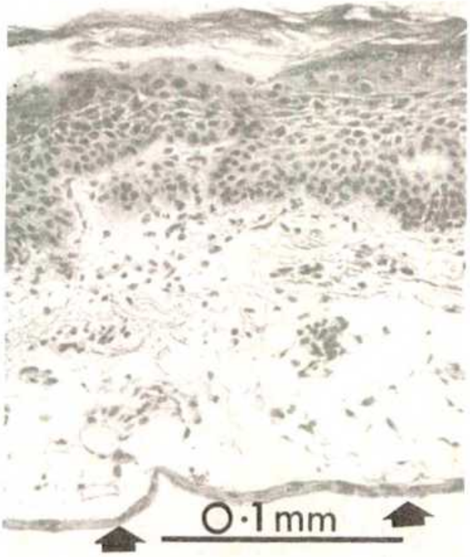

FIG. 1.—Control culture, showing migration of epidermis around the dermis (arrows).

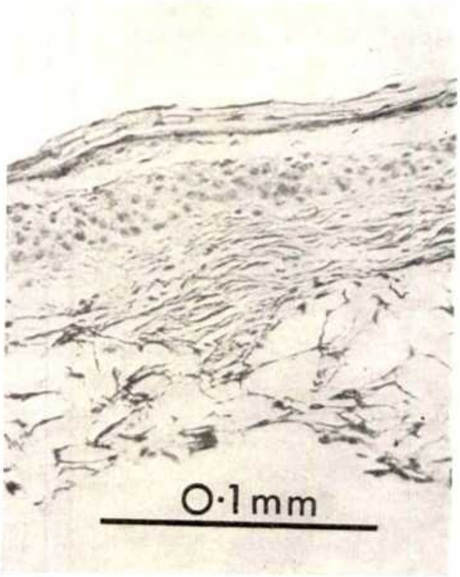

FIG. 2.—Organ culture treated with 5 μg/ml cytochalasin B. There has been no migration, but the cells appear healthy.

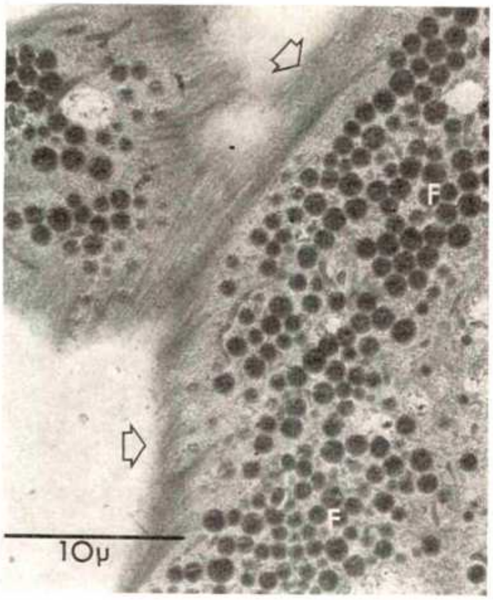

FIG. 3.—Control culture: low power view, showing the distribution of micro-filaments (arrows), fat droplets (F).

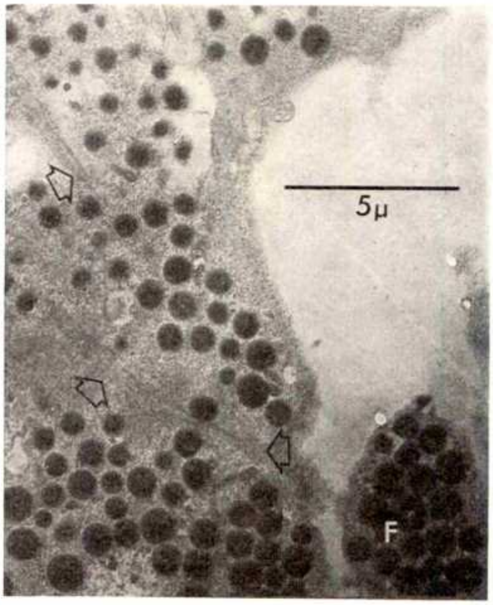

FIG. 4.—Cytochalasin B 20 μg/ml for 4 hours: low power veiw, showing distribution of microfilaments (arrows) after treatment.

added at final concentrations of 1, 2, 5 and 20 μg/ml. Control cultures were made in medium without any additive, and with quantities of DMSO equivalent to that in the experimental cultures. Sections were prepared and examined after staining with H. and E.

Monolayer cultures were prepared by a slight modification of the method described by Cruickshank *et al.* (1960). In brief, slices of guinea-pig ear skin were trypsinized and the epidermal cells incubated on the cover slips of special chambers. The chamber originally described has now been replaced by a disposable model (Nuclon Plastics Ltd.). The same medium was used as for the organ cultures. After the sheets had formed, time lapse cinefilm was taken before and after the addition of cytochalasin B.

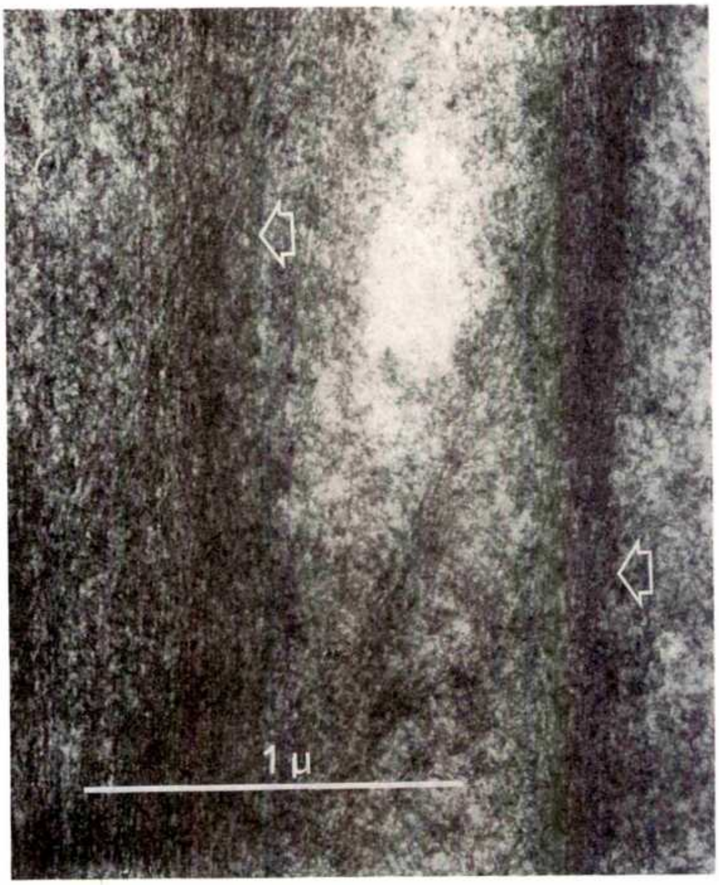

Fig. 5.—Control culture: high power view of microfilaments (arrows).

For electron microscopy, trypsinized epidermal cells were grown in Falcon flasks. All the monolayer cultures were fixed and dehydrated *in situ*. Most were processed as described by Constable (1972), while others were detached from the substrate with a small rubber policeman, centrifuged in capsules (Taab Labs) and embedded in Epon as pellets. The cells used in the experiments were grown for 29 or 31 days. In control and treated cells, between 10 and 40 cells were examined in each category.

RESULTS

At 1 μg/ml cytochalasin B had no effect on epiboly; several layers of cells covered the dermis in both control and treated cultures. At 2 μg/ml a slight effect was noticed. Whereas the controls behaved normally, migration in the treated cultures was limited to the edges of the explant and to the sites of cut hair follicles. At 5 μg/ml complete inhibition of migration occurred. A comparison of control and cultures grown in this concentration of cytochalasin B is shown in Fig. 1, 2. It will be noted that the original epidermal cells appear healthy. However, at 20 μg/ml, not only was migration prevented, but the epidermal cells died.

Time lapse studies showed that, at 5 μg/ml, there was no inhibition of the small amount of movement which normally takes place in epidermal cell cultures, and ruffling and intracellular movement continued at the usual rate. In one case a mitosis followed by normal separation of the daughter cells was seen, and in the same culture a division followed by reunion of the daughter cells was observed. Nuclear extrusion was not seen.

At 20 μg/ml ruffling and movement ceased, and the cytoplasm of the cell retracted, leaving only long fibrils extended on the glass. When the control culture medium was replaced, the cells spread and appeared to recover, but very large vacuoles were seen in the cytoplasm, indicating that recovery was not complete and that the medium was at least partially toxic. This concentration proved fatal to the cells after 18 hours' exposure.

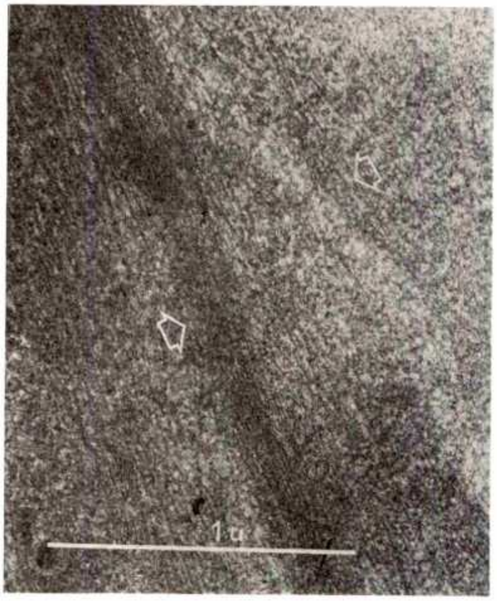

FIG. 6.—Cytochalasin B 20 μg/ml for 4 hours: high power view of cell after treatment: microfilaments (arrows).

The ultrastructural appearance of cells in control culture was essentially the same as that described by Constable (1972). Briefly, nuclei were indented and had prominent nucleoli; mitochondria were enlarged and often branched. There were many ribosomes, polyribosomes and more endoplasmic reticulum than is seen in whole skin; lysosomes and microtubules were both prominent. Large amounts of filamentous material were seen, presumably comprising both tonofilaments and the microfilaments which are seen in all cell types in culture. Those cells which were sectioned after pelleting were for this reason viewed in various different planes. Those cells sectioned in the plane of the monolayer showed more visible filaments than those cut in other planes. A further observation made in the pelleted cells and not seen in the previous study (Constable, 1972) of cells cut in the plane of the monolayer were the cup-shaped indentations described by Abercrombie *et al.* (1971) in fibroblasts sectioned vertically through the cell. They occurred in both control and cytochalasin-treated epithelial cells.

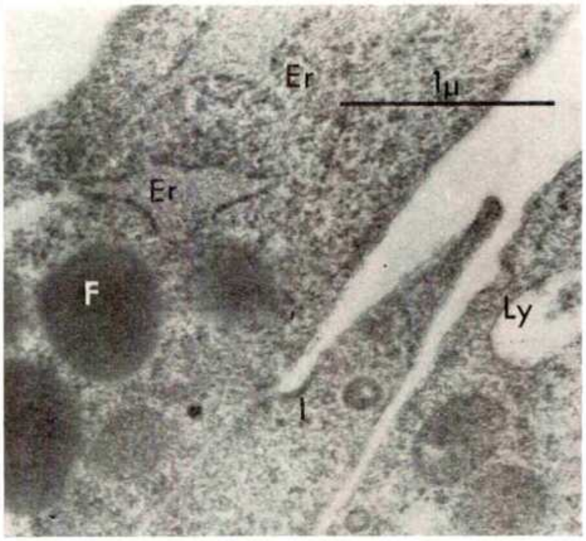

FIG. 7.—Cytochalasin B 5 μg/ml for 24 hours: the cell shows lysosomes (Ly) and dilated endoplasmic reticulum (Er), fat droplet (F), cup-shaped indentation (I).

Cells exposed to cytochalasin B at 5 μg/ml for 24 hours showed only a slight reduction in the microfilament system, although it was not prominent in either the treated or the untreated cultures. The number of lysosomes appeared to be increased in the treated cultures and the rough endoplasmic reticulum was dilated in places. Either of these may be responsible for the large vacuoles seen by phase contrast in the time lapse study. After 4 hours' treatment at 20 μg/ml, no convincing reduction in the amount of microfilaments could be seen. In both treated and control cultures the microtubular system was prominent and not adversely affected by cytochalasin B. Granular material was present in the cytoplasm of both treated and control cultures.

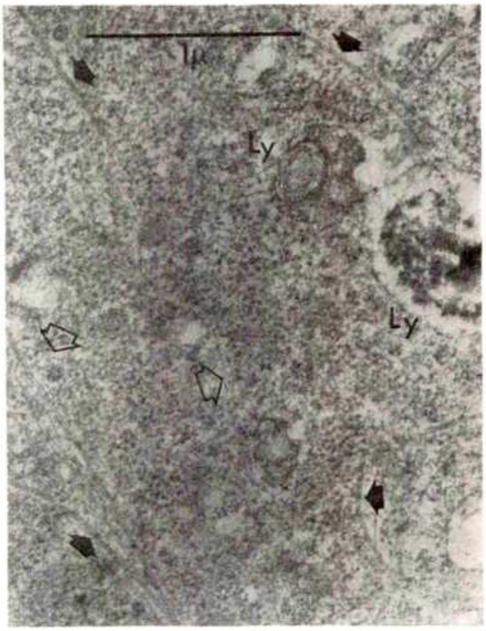

FIG. 8.—Cytochalasin B 5 μg/ml for 24 hours: the cell shows lysosomes (Ly), microfilaments (arrows). The microtubules (solid arrows) are very prominent.

DISCUSSION

Cytochalasin B is active in organ culture of skin, a system which is much closer to the *in vivo* situation than dispersed cell cultures. However, to inhibit cell migration, the concentration required is greater than that necessary to induce changes in fibroblasts. At 1 μg/ml no effect was observed on epidermal cells, but fibroblasts (Carter, 1967), macrophages (Allison *et al.*, 1971) and leucocytes (personal observation) are almost instantly halted at this concentration.

At 5 μg/ml the concentration which inhibited epiboly, internal cell movements in monolayer culture were not dramatically altered. In general epidermal cells in monolayer culture do not move for great distances. It may be that the more active the cell movement the more effective is the cytochalasin. At 5 μg/ml the adult epidermal cells in monolayer were undoubtedly affected because a division occurred followed by a reunion of the cytoplasm. Another cell in the same field, however, was able to divide normally, indicating that individual cells may have different thresholds of response to cytochalasin B.

The electron micrographs did not provide convincing evidence that the microfilament system was affected at low concentrations of cytochalasin B (5 μg/ml). The granular material seen was similar to that described in glial cells and oviduct (Wessells *et al.*, 1971) and may have been produced by the disruption of microfilaments, or possibly tonofilaments. The plane of sectioning may affect the apparent amounts of granular material, some of which may in fact represent filaments cut in cross section.

The retraction of cytoplasm and vacuolation seen at 20 μg/ml and 40 μg/ml were almost certainly general toxic effects, although a specific effect on the microfilament system may also have occurred. The cells died if left in medium containing this concentration of cytochalasin B for 18 hours, but they recovered if left for only 3 hours. Retraction of the cytoplasm and assumption of a stellate shape have been described in glial cells exposed to 7 μg/ml of cytochalasin B for times "later than 10–15 minutes" (Wessells *et al.*, 1971), and is probably also due to toxicity. Linville and Shepard (1972) make particular mention of the fact that the mortality was as high as 88% in chick embryos treated with a solution of 10μg/ml for 20–30 hours.

It would seem that, although cytochalasin B has specific effects on the behaviour of cells, there is also a toxic effect which has been greatly underestimated.

In general, therefore, although one can conclude that cytochalasin B can exercise effects on epidermal cells *in vitro*, both on organ cultures and primary dispersed cell cultures, these cells probably have a higher threshold, as the effects are less dramatic than on other types of tissue culture cells which have been grown *in vitro* for prolonged periods, and on cells which normally exhibit active movements.

We are greatly indebted to Mr J. R. Cooper for his assistance, particularly with the time-lapse cinephotomicrography.

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LIPID SYNTHESIS IN RAT SKIN

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Summary.—Rat dorsal skin was fractionated by means of an electrokeratotome into epidermis and dermis containing the sebaceous glands. These fractions were separately cultured in the presence of ^{32}P-orthophosphate, ^{14}C-acetate, ^{14}C-glycerol and ^{14}C-mevalonate, and the radioactivity incorporated into different classes of lipids was measured. The nature of the labelled products was different in the epidermis compared with the sebaceous glands: in the former, phospholipids and sterols were the major radioactive lipids synthesized, whereas, in the sebaceous glands labelled triglycerides, sterol esters and wax esters predominated.

The nature of individual fatty acids labelled from ^{14}C-acetate was studied and it was found that the sterol/wax esters fractions of both epidermis and sebaceous glands contained more radioactive odd-numbered normal chain and iso- and anteiso-branched chain fatty acids than the phospholipids and triglycerides. Isolated epidermal cells were prepared, and lipogenesis studies with radioactive precursors showed labelling patterns very similar to those of the cultured intact epidermis fractions. The presence of biochemically active sebaceous glands in the dermal residues, after excision of the epidermis fractions, was confirmed by treating skin *in vivo* with methyl testosterone, a hormone which stimulates the function of sebaceous glands, when a higher rate of lipogenesis was observed in the subsequently cultured dermal residue fractions, compared with untreated controls. No such differences were seen in the epidermis after testosterone treatment.

MEASUREMENT of lipid synthesis in skin *in vitro* has been described for numerous species, including human (Vroman *et al.*, 1969), guinea-pig (Wheatley *et al.*, 1970) and mouse (Brooks *et al.*, 1963; 1966; Prottey and Ferguson, 1972), but this technique has not been extended to rat skin in such detail, despite considerable data on the chemical nature of rat skin lipids (Clayton *et al.*, 1963; Horlick and Avigan, 1963; Nikkari and Haahti, 1964; Nikkari, 1965). Wilson (1963) described the synthesis of sterols from ^{14}C-acetate by rat skin cultured for short times. Similarly, Ziboh and Hsia (1969) demonstrated active metabolism of both ^{14}C-acetate and ^{14}C-glycerophosphate, although the precise identities of the lipid products were not described. Grigor *et al.* (1970) have demonstrated the synthesis of iso- and anteiso-branched fatty acids *in vivo* after injection of specific radioactive precursors.

Recently, in a comprehensive study of lipogenesis in cultured human skin, Hsia *et al.* (1970) have shown that various fractions of the whole tissue possessed different capacities for lipid synthesis: in particular, the sebaceous glands manufactured squalene and triglycerides, whereas greater proportions of sterols and polar lipids were synthesized in the epidermis. On a weight basis, the epidermis was several times more active than the dermis for lipogenesis. Also, Summerly

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and Woodbury (1971) have described very similar data for lipogenesis in sebaceous glands and appendage-free epidermis, separated by exposure to calcium chloride solutions. We have performed similar experiments with rat skin in culture, but using epidermis separated from the sebaceous glands by means of the keratotome slicing technique. The use of various radioactive precursors in the cultures has shown differences in the types of lipids synthesized in epidermis and dermis, with labelled phospholipids and sterols predominating in the former region, and labelled sterol esters, wax esters and triglycerides in the latter. The types of labelled fatty acids esterified to various lipid classes have also been studied; the sterol/wax esters containing more uncommon fatty acids (odd-numbered chain normal acids, anteiso- and iso-branched acids) compared with phospholipids and triglycerides. The validity of techniques for tissue fractionation have been confirmed for the epidermis by comparing lipogenesis in isolated epidermal cells with intact epidermis, and for the sebaceous glands by comparing the effect of methyl testosterone, which specifically affects sebaceous gland function.

MATERIALS AND METHODS

Reagents.—All chemicals and solvents used were of analytical grade of purity. Hexane for thin-layer chromatography was redistilled over potassium permanganate before use. The suppliers of certain specialized materials are recorded at the appropriate point in the text.

Animals.—All animals were female rats of the Colworth-Wistar Albino CD-inbred strain, aged 4–5 weeks, fed Spital diet and water *ad libitum*. Litter mates were not necessarily used.

Radioactive materials.—All were purchased from the Radiochemical Centre, Amersham. ^{32}P-orthophosphate, in dilute HCl, was neutralized with $NaHCO_3$ and diluted with sterile 0·9% saline such that 0·1 ml contained 52·5 μCi on the day of the experiments. The specific activity was 55 μCi/μmol. Acetic acid-2-^{14}C, sodium salt, was diluted with sterile $NaHCO_3$ solution such that 0·1 ml contained 5·0 μCi. The specific activity was 56 μCi/μmol. Glycerol-^{14}C(U) was diluted with sterile $NaHCO_3$ solution such that 0·1 ml contained 5·0 μCi, the specific activity being 13·3 μCi/μmol. D,L-mevalonic acid 2-^{14}C lactone in benzene solution, was layered on to sterile 5% $NaHCO_3$ solution and the benzene removed with N_2. The weak base hydrolysed the lactone ring, giving a clear solution of 2-^{14}C-D,L-sodium mevalonate. The volume was adjusted such that 0·1 ml contained 5·5 μCi, the specific activity being 5·85 μCi/μmol.

Preparation of epidermis and dermis.—Rats were killed by cervical dislocation and the dorsal skin clipped to remove hair. The shaved areas of skin were swabbed with 70% alcohol and then excised. Subcutaneous fat was trimmed off, if present. Slices were cut using a Castroviejo Electrokeratotome (Stortz Instrument Co., St. Louis, Missouri, U.S.A.), fitted with the 200 μm shim. Slices of the whole epidermis contained a small amount of the upper dermis, and generally had an average thickness of about 150 μm. The slices are referred to as the " epidermis " in the culture experiments described below. The tissue remaining after removal of the slice was termed the " dermal residue " or " dermis " in the subsequent culture experiments. The dermal residues were generally about 500 μm thick and contained hair follicles and sebaceous glands, the latter being situated as a planar band about 100 μm below the cut edge. Tissues prepared in this manner were then placed, upper surface downwards, in a sterile petri dish containing a gauze pad and 5 ml of a mixture of mycostatin (100 mg/l), dihydrostreptomycin sulphate (100 mg/l) and benzyl penicillin (2 × 10^5 units/l) in Hanks' phosphate buffered saline (Hanks and Wallace, 1949) for 30 min at 25°C. Mycostatin was obtained from E. R.

Squibb and Sons, Liverpool, dihydrostreptomycin sulphate BP and benzyl penicillin BP were obtained from Dista Products, Ltd., Liverpool.

Isolation of cells from rat skin epidermis.—200 μm keratotome slices cut from the skins of 10 rats; and, after treatment with antibiotics for 30 min, were incubated at 37°C for 35 min in 0·25% trypsin (Hopkin and Williams, Chadwell Heath, Essex) in Dulbecco's medium (Dulbecco and Vogt, 1954), which is a phosphate-buffered saline, minus Ca^{++} and Mg^{++} (Willmer, 1965). The epidermis was then separated from the underlying slivers of dermis by gentle teasing. The separated fragments of epidermis were then digested in 0·25% trypsin in Dulbecco's medium for 30 min at 37°C with gentle stirring. The solution became turbid due to release of cells. Two ml rat serum was then added to arrest protease activity. The resultant cell suspension was filtered through gauze, centrifuged at 400 *g* for 10 min at 4°C and the supernatant discarded. After resuspension in rat serum and recentrifugation, the cell pellet was resuspended in medium 199 (Morgan *et al.*, 1950) containing pencillin (200 i.u./ml), streptomycin (100 i.u./ml), heparin (10 i.u./ml) and 20% (v/v) rat serum, the dilution being such that 1 ml contained accurately about 2×10^6 cells (determined by means of a Hawksley Crystalite Counting Chamber, Lancing, Sussex).

Culture in vitro *of tissues and cells (a) Tissues.*—200 μm thick keratotome slices and the underlying dermal residues were cultured in vessels recently described by Friend (1972). These vessels were sterilized by autoclaving at 15 lb/inch² for 20 min prior to use.

The fragments of tissue for culture (generally one piece, measuring 8 mm × 5 mm for the epidermis; 4 mm × 3 mm for the dermis) were placed on the mesh and the well filled to the level of the mesh by 1·0 ml of the culture medium. In all experiments the culture medium comprised 0·1 ml of the requisite solution of radioactive precursor +0·9 ml Waymouth's medium MB 752/1 (Waymouth, 1959) containing 10% (v/v) calf serum. To maintain a high level of humidity during culture 1·0 ml sterile distilled water was placed on the filter paper beneath each culture vessel. The petri dishes containing the tissue fragments were then placed in a desiccator fitted with a gas line which continually delivered a stream of moist gas (a mixture of air +5% CO_2). The desiccators were maintained for culture at 37°C.

Culture in vitro *of tissues and cells (b) Cells.*—Portions (0·9 ml) of the cell suspensions in medium 199 + 20% (v/v) rat serum were added to sterile Leighton tubes, together with 0·1 ml of the requisite radioactive precursor solution. The tubes were briefly gassed with 5% CO_2 in air, stoppered with sterile bungs, and cultured at 37°C.

Extraction of radioactive lipids (a) Tissues.—After culture the tissues were removed from the vessels, extensively rinsed with ice-cold 0·9% saline and homogenized in 5·0 ml trichloroacetic acid (5% w/v). The homogenates were centrifuged and the pellets extracted overnight with a total of 8·0 ml chloroform-methanol (2 : 1 w/v) to extract the lipids. The pellets were removed by centrifugation, and 1·6 ml 0·5mol/l potassium phosphate buffer, pH 7·4, containing KCl (2mol/l) was added. The phases were vigorously mixed and, after centrifugation, the upper (aqueous) phase was discarded. All lower phases were rewashed with equal volumes of "synthetic upper phase" (made by mixing methanol, the phosphate buffer solution and chloroform in the proportions 48 : 47 : 3), and the upper phases again discarded. This procedure, which removes water-soluble, non-lipid contaminants from the lipid extracts, was termed Folch washing (Folch *et al.*, 1957). The lower (chloroform) phases were then made up to standard volume for determination of radioactivity, and subsequently analysed by thin-layer chromatography.

Extraction of radioactive lipids (b) Cells.—To the 1·0 ml cell cultures after culture was added 5·0 ml chloroform-methanol (2 : 1 v/v) and these were left at room temperature overnight for extraction of lipids. After centrifugation, the upper phases were discarded, together with the layer of proteinaceous material at the interphase. The organic layers were then re-extracted with equal volumes of " synthetic top phase ", as above. After dilution to standard volume for determination of radioactivity, the lipids were studied by thin-layer chromatography.

Thin-layer chromatography.—All separations were achieved using 0·25 mm layers of silica gel H (E. Merck AG, Darmstadt, W. Germany). Phospholipids were separated using solvent mixture A: chloroform : methanol : glacial acetic acid : water (170 : 30 : 20 : 7·2, by vol.). For 2-dimensional separations of phospholipids the plates were developed firstly in chloroform : methanol : 880 ammonium hydroxide (65 : 35 : 5, by vol.), followed by solvent A in the second dimension. Different lipid classes were separated using solvent B: hexane : diethylether : 90% formic acid (170 : 30 : 2, by vol.). This solvent system did not separate squalene from sterol/wax esters. However, a further development in solvent C, pure hexane, effected a separation of the former from the latter 2. Sterol esters and wax esters are inseparable on thin layers of silica gel. Individual types of sterol were separated, using solvent D-hexane : diethylether (65 : 35, v/v), a double development being necessary to separate lanosterol from cholesterol. Lipids were localized by exposure of the plates to iodine vapour and identified by reference to standard compounds. Positive identification of the water-soluble products of deacylated phospholipids was achieved by the base deacylation—paper chromatographic method described by Prottey and Hawthorne (1966).

Gas-liquid radiochromatography.—Quantitative analysis of radioactive fatty acid methyl esters was performed on a chromatograph, as first described by James and Piper (1963) and modified by James and Hitchcock (1965). Mass detection was by catharometer, and radioactivity by means of a proportional counter. The column was 7 ft × $\frac{1}{4}$ inch (1·8 metre × 6·3 mm) i.d. copper tube, the stationary phase being 10% FFAP on celite (80–100 mesh). To aid identification of the radioactive peaks, carrier fatty acid methyl esters were added to the radioactive samples. In addition, identification of peaks by means of their " carbon numbers " (Woodford and Van Gent, 1960) was carried out. Quantitation of the radioactive peaks was achieved by triangulation. Fatty acids were methylated by reflexing in methanolic sulphuric acid.

Measurement of radioactivity.—Radioactivity was measured with a Packard Tri-carb 4322 liquid scintillation spectrometer, using a dioxane-based phosphor. Counting efficiencies were determined by means of an external standard. Radioactive areas on thin-layer plates were localized by dividing the developed plate into 0·5 cm bands and scraping the gel into scintillation counting vials, followed by radioactivity counting.

Treatment of rats with methyl testosterone.—Female rats aged 3 weeks were clipped to remove hair from their dorsal skin, and then treated topically, twice per day, with 0·1 ml of a mixture of 10% methyl testosterone in petroleum jelly, for a total of 5 days. At the end of treatment the animals were left a further 9 days to allow the sebaceous glands to respond to treatment. Control animals were similarly treated, but using 0·1 ml petroleum jelly alone. The methyl testosterone solution was made by dissolving 2·5 ml petroleum jelly in 5·0 ml distilled hexane, with warming. To this was added 250 mg 17(α)-methyl ^{4}-androsten-17(β)-ol-3-one (methyl testosterone, Sigma, St. Louis, Missouri) dissolved in acetone. The solvents were evaporated under nitrogen, with warming, and the preparation stored in a vacuum desiccator. After 14 days had elapsed from the first treatment, the animals were killed, and any growth of hair removed by clipping. The skin was excised and 200 μm keratotome slices of epidermis and the dermal residue cultured as described above.

DNA determination.—This was by the modified method of Burton (1968), using calf thymus DNA (Sigma) as a standard.

RESULTS

Composition of epidermal and dermal skin fragments obtained by keratotome sectioning.—Figs. 1a and 1b show respectively an epidermal slice and the corresponding dermal residue. The 200 μm thick slice contained the whole of the epidermis, together with a small amount of the upper corium, but no sebaceous glands or hair bulbs, these appendages being wholly contained in the dermal residue.

Viability of rat skin cultured in vitro.—The method for studying lipid synthesis in rat skin involved culture of tissues in the presence of specific precursors of lipids. This technique has been employed by others, and has the advantage of

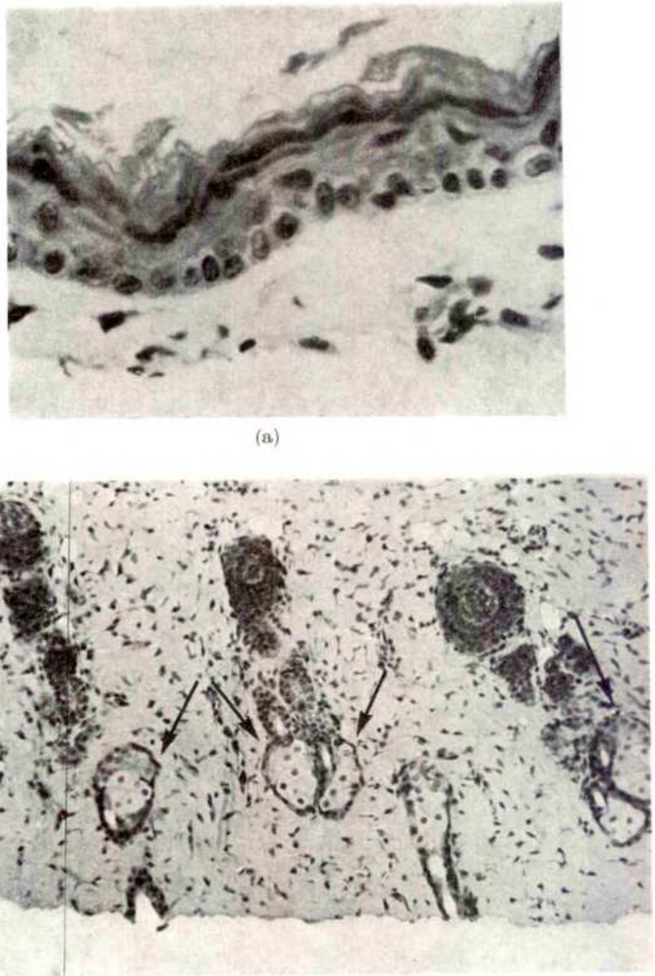


FIG. 1.—(a) 200 μm thick keratotome slice of rat skin epidermis (H. and E. × 533). (b) Dermal residue of rat skin after excision of a 200 μm thick epidermal slice. The sebaceous glands are shown by arrows (H. and E. × 533).

obviating the influences of the circulatory system, hormones etc., compared with lipogenesis studies *in vivo*. In preliminary experiments on the long-term culture of rat skin we were unable to maintain the viability of the tissues for periods of time approaching 48 hours or longer: general necrosis of the Malpighian layer and sebaceous glands were commonly observed, regardless of modifications to the culture media, temperature of culture or gas phase.

In order to show that rat skin in culture was viable for periods of at least 24 hours, the experiment illustrated in Fig. 2 was performed. For each precursor studied the culture rings contained rectangles of tissue (either 200 μm thick keratotome slices of epidermis, or the corresponding underlying dermal residue, in which the sebaceous glands were situated) in the presence of 1·0 ml culture medium. In the ^{32}P-orthophosphate incorporation into phospholipids, 52·5 μCi of the labelled precursor was added; for 2-^{14}C-acetate incorporation into total lipids 5·0 μCi of precursor was added. With both precursors studied epidermal radioactivity increased linearly with time. With the dermal fraction there was linear uptake of ^{32}P-orthophosphate into phospholipids over 24 hours, but with ^{14}C-acetate the rate at 24 hours of culture was falling off. This may be due to impairment of the functioning of the sebaceous glands after 24 hours in culture (histological examination of similarly cultured tissues has sometimes indicated cellular necrosis in sebaceous glands after 24 hours of culture), or to dilution of the ^{14}C-acetate in the medium, by uptake into the cultured skin, or to conversion of the exogenous glucose in the medium to unlabelled acetate. Fig. 2 shows, however, that the cultured skin fragments actively incorporated both types of lipid precursor during 24 hours at 37°C. On the basis of this, all subsequent tissue cultures were carried out for this time period.

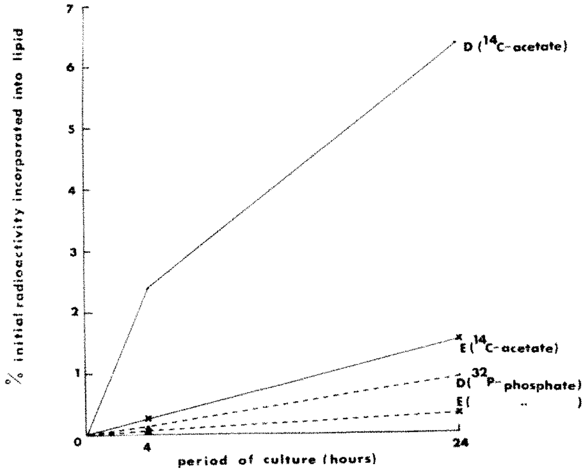


FIG. 2.—Incorporation of ^{32}P-orthophosphate (---) and ^{14}C-acetate (—) into lipids of rat skin epidermis and sebaceous glands during culture *in vitro* for 24 hours.

Nature of phospholipids labelled during culture of rat skin in vitro *in the presence of ^{32}P-orthophosphate.* The lipids derived from the experiment of Fig. 2, using ^{32}P-orthophosphate as a precursor, were chromatographed on silica gel, using solvent system A; standard phospholipids were chromatographed to aid identification. The distribution of radioactivity on the chromatograms are shown in Table I. Positive identification of the major labelled phospholipids was achieved by deacylation of the total lipid mixtures, followed by paper chromatography of the resultant glycerylphosphoryl derivatives. Radioactivity was detected in the

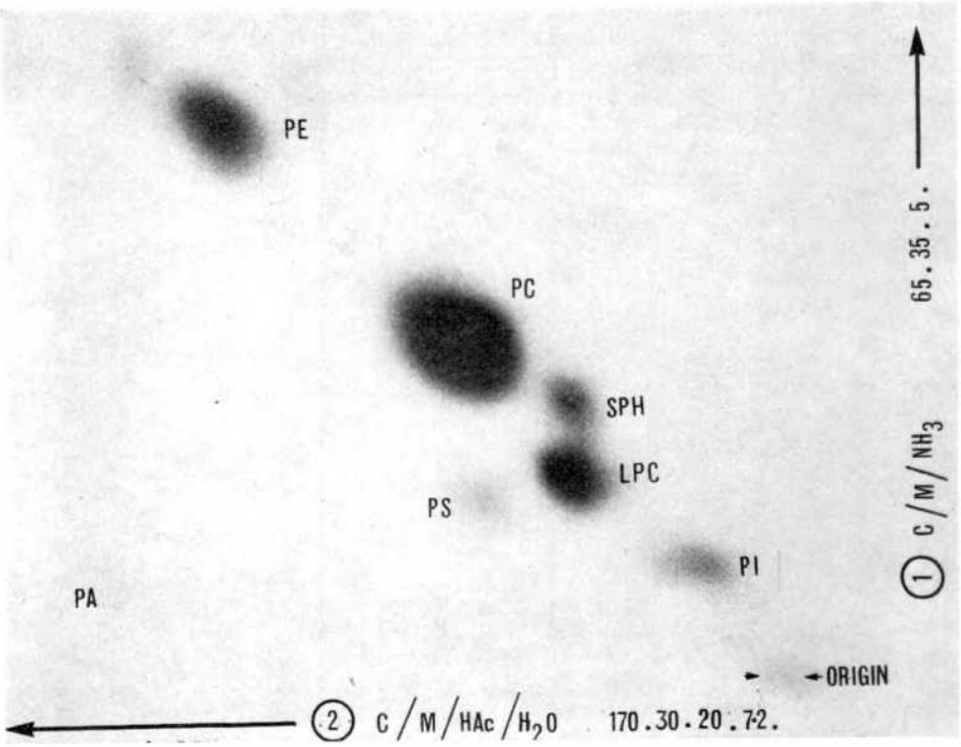


FIG. 3.—Autoradiogram of phospholipids of epidermis, labelled during 24-hour culture *in vitro* in the presence of ^{32}P-orthophosphate. Abbreviations: PE, phosphatidylethanolamine; PC, phosphatidylcholine; SPH, sphingomyelin; LPC, lysophosphatidylcholine; PS, phosphatidylserine; PA, phosphatidicacid, and PI, phosphatidylinositol.

TABLE I.—*Phospholipids of Rat Skin *Epidermis and *Dermis, Labelled During Culture in the Presence of ^{32}P-orthophosphate*

	Radioactivity (% of total lipid radioactivity)			
	4-hour culture		24-hour culture	
Phospholipid	Epidermis	Dermis	Epidermis	Dermis
Phosphatidic acid	0·2	0·2	6·9	2·3
Phosphatidylethanolamine	17·0	21·8	19·6	16·8
Phosphatidylcholine	57·6	59·0	47·2	57·2
Inositol, serine and lysophospholipids + sphingomyelin	25·2	18·9	26·3	23·7

*In this context, and in Tables II–VII, epidermis means a 200 μm thick keratotome slice, and dermis means the dermal residue beneath the site of excision of the slice.

following compounds: α-glycerophosphate (from phosphatidic acid); glycerylphosphorylcholine (from phosphatidylcholine); glycerylphosphorylethanolamine (from phosphatidylethanolamine); glycerylphosphorylinositol (from phosphatidylinositol) and glycerylphospherylserine (from phosphatidylserine). In addition, each lipid sample studied, small amounts of radioactivity, generally less than 10% of the total, remained lipid-soluble after base deacylation. This was presumably sphingomyelin, which is resistant to such hydrolysis. Two-dimensional thin-layer chromatography was also used, and Fig. 3 shows an autoradiogram of epidermal ^{32}P-labelled phospholipids after 24 hours of culture. In both epidermis and dermis, during 4 hours and 24 hours of culture, phosphatidylcholine was the major labelled phospholipid, representing 50% of the total lipid activity. Phosphatidylethanolamine generally contributed up to about 22% of the lipid radioactivity, whereas all other phospholipids were present in much lower proportions. Only traces of labelled phosphatidic acid were detected at 4 hours, this being somewhat higher at 24 hours. There were no distinct differences in the labelling pattern of the epidermal phospholipids and those of the dermis.

Nature of the lipid classes labelled during culture of rat skin in vitro *in the presence of* 2-^{14}*C-acetate.*—The radioactive lipids from the 24-hour cultures of epidermis and dermis in the presence of ^{14}C-acetate described in Fig. 2 were chromatographed on thin layer by double-development in solvent mixtures B and C. The distribution of radioactivity, summarized in Table II, showed that the acetate had been incorporated into both fatty acyl chains and into sterols. There were characteristic differences between epidermis and dermis. Phospholipids and unesterified sterols comprised nearly 70% of the total epidermal labelled lipids. There was almost 16% of the label in triglycerides, but sterol/wax esters and squalene were labelled to only an insignificant extent. The dermal labelled lipids, derived principally from the sebaceous glands, were rich in labelled sterol/wax esters, but phospholipids and free sterols were labelled to a lesser degree. Labelled free fatty acids were low in both regions, and the unidentified products probably contained partial glycerides, long-chain alcohols and diols.

TABLE II.—*Lipids of Rat Skin Epidermis and Dermis Labelled During Culture for 24 Hours in the Presence of* 2-^{14}*C-acetate*

	Radioactivity (% of total lipid radioactivity)	
Lipid class	Epidermis	Dermis
Combined phospholipids	32·7	12·7
Sterols	35·7	9·8
Unesterified fatty acids	6·6	3·5
Triglycerides	15·9	23·3
Sterol/wax esters	2·7	43·3
Squalene	0·4	0·3
Unidentified lipids	6·0	7·1

Individual fatty acids synthesized by rat skin epidermis and dermis during culture in the presence of 2-^{14}*C-acetate.*—In order to obtain higher levels of incorporation into lipids for detailed radiochromatographic analyses, skin pieces were cultured for 24 hours in the presence of 15 μCi 2-^{14}C-acetate. The epidermal cultures comprised of 4 fragments of 200 μm thick keratotome slices, measuring 8 mm × 5 mm, and the dermal cultures of 4 fragments measuring 4 mm × 3 mm. After culture, labelled lipids were extracted as described above and separated by thin-layer chromatography.

Individual lipid classes were recovered from thin-layer plates and transmethylated. Gas-liquid radiochromatographic separation of the fatty acid methyl esters gave the radioactivity patterns shown in Table III. In both the epidermis and dermis palmitic acid was the principal labelled fatty acid synthesized from acetate. Labelled palmitoleic, stearic acid and oleic acid were also predominant in phospholipids and triglycerides, although the sterol- and wax esters of both epidermis and dermis contained much lower proportions of radioactivity in the 2 C18 acids. Anteiso-branched odd-numbered chain acids and iso-branched even-numbered chain acids were labelled to a significant extent, especially in the sterol/wax esters fraction of both skin regions. For the epidermal sterol/wax esters they comprised a total of 20·7% of all acids synthesized; in the dermis this was 17·0%. The proportions of these acids in phospholipids and triglycerides, however, were much lower. No labelled fatty acids of chain length longer than C18 were detected in any of the lipids examined. Similarly, labelled octadecadienoic acid was not detected.

TABLE III.—*Labelled Fatty Acid Patterns of Lipids Synthesized in Rat Skin Epidermis and Dermis During Culture for 24 hours in the Presence of 2-^{14}C-acetate*

Fatty acid	Carbon number	Epidermis			Dermis*	
		PL	TG	S-/WE	PL	S-/WE
14 : 0	14·0	4·9	7·7	7·4	2·9	4·3
14 : 1	14·2	—	—	1·6	2·4	trace
Anteiso 15 : 0	14·65	—	—	3·1	—	3·4
15 : 0	15·0	2·0	1·4	3·0	1·2	3·2
Iso 16 : 0	15·5	1·6	6·3	6·6	—	7·2
16 : 0	16·0	42·7	48·1	40·6	53·2	32·6
16 : 1	16·2	16·8	8·7	10·7	13·6	31·5
Anteiso 17 : 0	16·65	—	—	6·1	—	4·4
17 : 0	17·0	1·7	2·8	2·1	—	2·9
17 : 1	17·2	1·2	—	1·1	—	trace
Iso 18 : 0	17·5	—	2·2	3·9	—	2·0
18 : 0	18·0	16·7	9·6	6·2	16·9	1·6
18 : 1	18·2	12·6	13·2	11·8	10·1	6·8

Figures represent % of total radioactivity recovered from column.
Abbreviations: PL, phospholipids; TG, triglycerides; S-/WE, sterol esters plus wax esters.
*Sample of dermal triglycerides was not examined.

Nature of glycerolipids labelled during culture of rat skin in vitro *in the presence of U-^{14}C-glycerol.*—Since it was clear from the studies using ^{32}P-orthophosphate and 2-^{14}C-acetate as precursors that glycerolipids were actively synthesized in both epidermis and dermis, and at different rates, similar fragments of rat epidermis and dermis were cultured in the presence of U-^{14}C-glycerol. Each culture vessel contained 2 fragments of the requisite tissue in contact with 1·0 ml culture medium, to which had been added 5·0 μCi U-^{14}C-glycerol. After culture for 24 hours, the radioactive lipids were extracted and separated by thin-layer chromatography in the solvent systems B and C. The distribution of radioactivity within the different lipid classes is shown in Table IV. Phospholipids and triglycerides contained approximately 70% of the total lipid radioactivity in both the epidermis

TABLE IV.—*Lipids of Rat Skin Epidermis and Dermis Labelled During Culture for 24 hours in the Presence of U-^{14}C-glycerol*

	Radioactivity (% of total lipid radioactivity)	
Lipid class	Epidermis	Dermis
Total phospholipids	55·8	21·9
Sterols	17·9	5·4
Unesterified fatty acids	1·1	2·9
Triglycerides	17·9	42·3
Sterol/wax esters	1·3	15·9
Squalene	0·4	0·3
*Unidentified	5·6	11·3

*The unidentified lipids included mono- and diglycerides, which were not separable from slow-moving lipids (e.g. sterols) in solvent mixture B.

and dermis, although the relative distribution of radioactivity between these lipid classes in the 2 morphologically distinct regions was striking. In the epidermis there was about 3 times the labelling in phospholipids (55·8%) compared with triglycerides (17·9%); whereas, in the dermis, the trend was reversed, with twice the activity in triglyceride (42·3%) as in phospholipids (21·9%). Another interesting finding was that many other lipid classes contained radioactivity, namely, free and esterified sterols, wax esters and free fatty acids, indicating that a portion of the added ^{14}C-glycerol had been metabolized, presumably to acetate units, and subsequently incorporated into fatty acyl and sterol moieties. To confirm this, a portion of the radioactive dermal triglyceride fraction was hydrolysed with aqueous NaOH and the resultant lipid-soluble and water-soluble products analysed for radioactivity. It was found that 15% of the initial triglyceride radioactivity was located in fatty acids, corroborating that ^{14}C-glycerol had been metabolized to other compounds as well as incorporated *per se* into glycerolipids.

Nature of lipids labelled during culture of rat skin in the presence of 2-^{14}C-D,L-mevalonic acid.—In order to establish the types of sterols which were labelled in rat skin epidermis and dermis, respective fragments from each region (one 8 mm × 5 mm fragment of a 200 μm keratotome section for the epidermis; one 4 mm × 3 mm fragment from the dermal residue) were cultured for 24 hours in the presence of 5·0 μCi 2-^{14}C-D,L-mevalonic acid. The radioactive lipids were extracted and

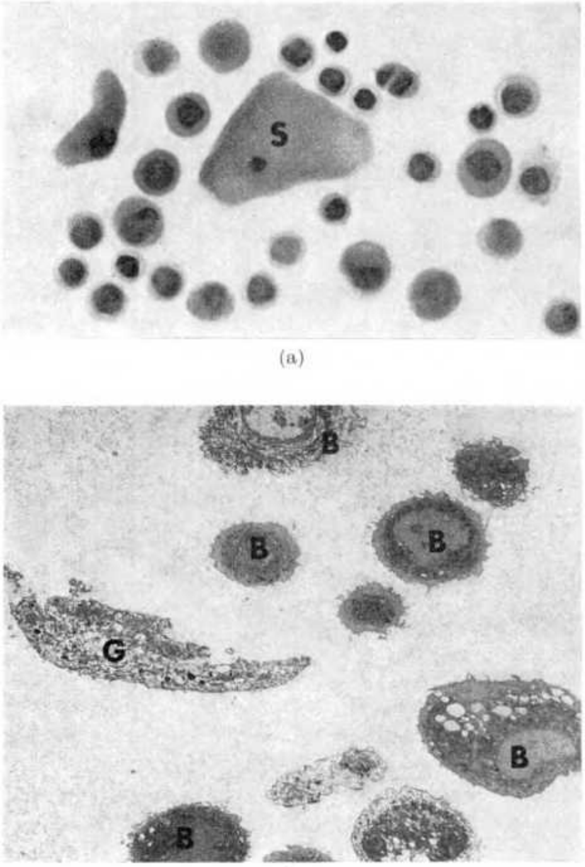
TABLE V.—*Lipids of Rat Skin Epidermis and Dermis Labelled During Culture for 24 Hours in the Presence of 2-^{14}C-D,L-mevalonic Acid*

	Radioactivity (% of total lipid radioactivity)	
Lipid class	Epidermis	Dermis
*" Phosphorylated intermediate "	16·0	10·0
Cholesterol	50·1	40·4
Lanosterol	13·4	9·9
Sterol esters	1·8	4·0
Squalene	3·3	24·3
†Unidentified	15·4	11·4

*Tentatively identified as farnesyl pyrophosphate. Remained at the origin on thin-layer chromatography.

†Possibly including polyisoprenoid compounds.

chromatographed upon thin layer in solvent systems B, followed by C, to separate individual lipid classes. Different types of sterol were separated by rechromatography in solvent D (double development). The radioactivity pattern is shown in Table V. In both epidermis and dermis free sterols (cholesterol and lanosterol) contained more than 50% of the total lipid radioactivity; however, the dermis contained far more radioactive squalene (24·3%) than the epidermis (3·3%). Both regions of the skin showed very meagre labelling in sterol esters, this being in contrast to the data shown in Tables II and IV, where the dermis, in particular, contained large amounts of radioactivity in this lipid class when other lipid precursors were used. This suggests that, under these culture conditions, sterol esters incorporate very little radioactivity from acetate into the sterol moiety, but rather, the radioactivity is associated with the acyl chains. Some lipids described in Table V were not characterized. These included a very polar com-



(a)

(b)

FIG. 4.—(a) Epidermal cells isolated by trypsinization of keratotome sections of rat skin. The cell harvest included basal cells, differentiated cells and complete squames (S) (H. and E. × 800). (b) Electron micrograph of isolated epidermal cells. Cells of the stratum basale predominated (B), with occasional cells of the stratum granulosum (G) (× 2800).

pound (or compounds) which remained at the origin of the chromatograms. This, presumably, was an intermediate in the conversion of mevalonic acid to squalene, because preliminary experiments at shorter times of culture showed more of this compound than squalene compared with the 24 hour cultures described here. Its behaviour on thin-layer chromatography in more polar solvent systems suggested that it was farnesyl pyrophosphate. The unidentified lipid which contained 15·4% and 11·4% of the radioactivity in the epidermal and dermal lipid respectively was possibly a polyisoprenoid alcohol. This radioactive component had a mobility on thin layer (solvent B) intermediate between free sterols and esterified sterols.

Lipid synthesis in isolated epidermal cells of rat skin in vitro.—It has been assumed in the experiments of Table I–V that the 200 μm thick keratotome slices contained the whole of the epidermis, but was free from contaminating sebaceous glands, which were located exclusively in the dermal residues. This assumption was based on light microscopy data, as illustrated in Figs. 1a and 1b. To corroborate the validity of this assumption, epidermal cells were prepared by controlled trypsinization, cultured in the presence of a variety of lipid precursors, and the results compared with those obtained for cultured intact epidermis. The cells prepared in this way were not exclusively those of the stratum basale, but, as may be seen in Figs. 4a and 4b, were contaminated with squames, differentiated cells of the stratum granulosum and possibly cells from the upper regions of hair follicles. The number of cells studied in the ^{32}P-orthophosphate experiment was $1{\cdot}85 \times 10^6$ in 1·0 ml culture medium, and these were incubated with 5·25 μCi ^{32}P-orthophosphate at 37°C for 3 hours and 27 hours. In the experiments using ^{14}C-labelled precursors, each culture contained $2{\cdot}33 \times 10^6$ cells per 1 ml of medium, the respective amounts of precursors being 5·0 μCi 2-^{14}C-acetate, 5·0 μCi U-^{14}C-glycerol and 5·0 μCi 2-^{14}C-D,L-mevalonic acid. These were cultured at 37°C for 4 hours and 24 hours. After culture, the radioactive lipids were extracted, purified by salt-washing and chromatographed on thin layers, using a variety of solvent systems similar to those described for the epidermal and dermal tissue cultures. The distributions of radioactivity within the various lipids are shown in Table VI.

For the cell cultures with ^{32}P-orthophosphate as a precursor, the patterns of labelling were quite similar at the 2 times studied. At 27 hours phosphatidylcholine was the major radioactive phospholipid, followed by phosphatidylethanolamine. The mixture of phosphatidylinositol, phosphatidylserine and sphingomyelin were not separable by unidimensional thin layer chromatography. The patterns obtained from the cell culture experiment were strikingly similar to those shown by the epidermal slice cultures of Table I.

In the ^{14}C-acetate cell cultures, at 4 hours phospholipid radioactivity accounted for 50% of the total, and free sterols for about 22%. At 24 hours, however, the proportion of the former had decreased, and that of the latter increased, this probably being a reflection of the relative rates of synthesis and/or turnover of these types of lipid. Again, phosphatidylcholine was the major labelled phospholipid. Triglyceride radioactivity was relatively low, about 15% of the total at both 4 hours and 24 hours. No sterol/wax ester labelling was detected, and only a trace of radioactive squalene, the latter being at 4 hours only. When these data are compared with Table II; it is seen that the 24-hour cell culture lipid radioactivities were very similar to those of the intact epidermis, but quite unlike

TABLE VI.—*Radioactive Lipids of Rat Epidermal Cells After Culture in the Presence of Various Precursors*

	% of total radioactivity incorporated into lipid during culture							
	^{32}P-orthophosphate		2-^{14}C-acetate		U-^{14}C-glycerol		2-^{14}C-D,L-mevalonic acid	
Lipid	3 hr	27 hr	4 hr	24 hr	4 hr	24 hr	4 hr	24 hr
Inositol and serine phospholipids + sphingomyelin	27·5	25·7	—	3·5	—	—	—	—
Phosphatidylcholine	62·1	49·9	29·4	20·7	62·8	46·7	—	—
Phosphatidylethanolamine	6·2	—	5·0	6·7	19·1	13·9	—	—
Phosphatidic acid	4·2	2·1	16·4	10·6	2·2	2·5	—	—
Total phospholipids	100·0	93·4	50·8	41·5	84·1	63·1	—	—
" Phosphorylated intermediate "(a)	—	—	—	—	—	—	84·7(a)	30·6(a)
Cholesterol	—	—	—	—	—	—	7·0	45·6
Lanosterol	—	—	—	—	—	—	3·8	10·9
Total free sterols	—	—	21·8	28·6	—	—	10·8	56·5
Free fatty acids	—	—	12·5	14·7	—	—	—	—
Triglycerides	—	—	14·0	15·3	15·9	36·9	—	—
Sterol esters + wax esters	—	—	—	—	—	—	—	—
Squalene	—	—	0·9	—	—	—	3·5	6·5
Unidentified lipids	—	6·6(b)	—	—	—	—	1·0(c)	6·4(c)

(a) Found only in ^{14}C-mevalonic acid experiments, tentatively identified as farnesyl pyrophosphate.
(b) An uncharacterized phospholipid.
(c) Tentatively identified as polyisoprenoid alcohol.

those of the dermis. Isolated epidermal cells cultured *in vitro* with ^{14}C-acetate thus exhibit the same pattern of lipid turnover as the epidermis *per se*.

The cells cultured in the presence of ^{14}C-glycerol again showed that phosphatidylcholine was the highest labelled phospholipid. After 4 hours of culture, 75% of the phospholipid radioactivity was found in this lipid. At 24 hours the proportions of both phosphatidylcholine and total phospholipid radioactivity had fallen: conversely, triglyceride radioactivity had increased, from about 16% at 4 hours to 37% at 24 hours. This, again, probably reflected the relative rates of synthesis and turnover of the 2 lipid classes. When these data are compared with those shown in Table IV for the whole epidermis culture, one sees that the level of labelled triglyceride was far higher in the isolated cells than that shown by the intact epidermis. Thus, the relationship between isolated cells and parent tissue is not so obvious as that for the ^{14}C-acetate experiment.

In the 4-hour cell culture with ^{14}C-mevalonic acid, the principal labelled lipid (84·7%) was a polar compound which remained at the origin after thin-layer chromatography in solvent B, and is referred to above as the " phosphorylated intermediate ", being tentatively identified as farnesyl pyrophosphate. Labelled cholesterol, lanosterol, squalene and an unidentified lipid were also seen, but in very small amounts. After 24 hours of culture, however, the labelling pattern was very different. The radioactivity in the " phosphorylated intermediate " had fallen to 30%, and there was a large increase in the proportion of labelled cholesterol (from 7% after 4 hours to 45·6% at 24 hours). The increases in radioactivity seen in lanosterol and squalene were much smaller. Such findings are in accordance with the accepted pathways (see Discussion) for sterol synthesis from mevalonic acid, where farnesyl pyrophosphate, squalene and lanosterol are intermediates in the formation of cholesterol. No sterol ester radioactivity was seen. The unidentified lipid increased by a factor of 6 during 24 hours, compared with the 4-hour culture value. This was also only tentatively identified, as a polyisoprenoid compound, which would be formed directly by polymerization of mevalonic acid units. When the data of Table VI are compared with those of Table V for whole epidermis, close similarities are seen. Both whole epidermis tissue cultures with ^{14}C-mevalonic acid and the isolated epidermal cell cultures differed from the dermal residue cultures in that squalene radioactivities were very much lower.

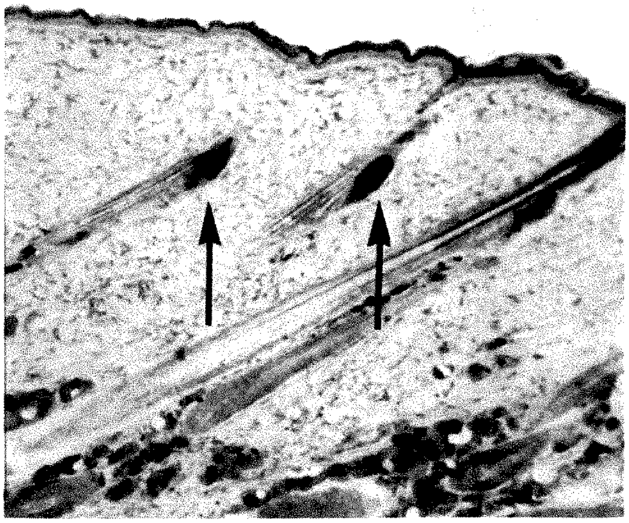
Corroboration that the dermal residue lipogenic activity is due to the sebaceous glands.—Fig. 1b shows that the dermal residue, after removal of a 200 μm thick epidermal slice, contains sebaceous glands and the cells of their associated hair follicles. In preliminary experiments we attempted to isolate intact sebaceous glands from the dermis by various chemical means (see Discussion), but we were consistently unsuccessful. Since topical treatment of human skin with the sex hormone testosterone causes the sebaceous glands to increase in both size and, ultimately, in excretion of sebum on to the skin surface (Strauss and Pochi, 1963), 200 μm thick keratotome slices of epidermis and the dermal residues remaining from rats which had received topical methyl testosterone were prepared. Fig. 5b shows whole skin from a testosterone-treated rat, and a dramatic increase in the size of the sebaceous glands is obvious, when compared with control (jelly only) skin (Fig. 5a). No changes were apparent in the epidermis, however. The epidermal slices and dermal residues were cultured in the presence of 5·0 μCi 2-^{14}C-acetate and 5·0 μCi U-^{14}C-glycerol for 24 hours at 37°C. Each culture

TABLE VII.—*Effect of Topically-applied Methyl Testosterone upon Lipid Synthesis in Rat Skin Fractions* In Vitro

Lipid radioactivity	Control skin			Testosterone treated skin		
		DNA content			DNA content	
	Lipid radioactivity % incorporation dpm	of tissue (ug)	Specific activity (dpm/ug DNA)	Lipid radioactivity % incorporation dpm	of tissue (ug)	Specific activity (dpm/ug DNA)
^{14}C-acetate culture						
(a) Epidermis	1·52; 167,140	47	3556	2·55; 280,380	81	3461
(b) Dermis	1·92; 210,960	121	1743	7·32; 804,780	132	6097
^{14}C-glycerol culture						
(a) Epidermis	0·64; 70,160	51	1376	0·66; 72,800	59	1234
(b) Dermis	1·73; 190,360	127	1499	2·69; 295,820	144	2054

vessel contained one fragment of tissue, for the epidermis, measuring 8 mm × 5 mm, and, for the dermis, 4 mm × 3 mm, in contact with 1·0 ml culture medium. Table VII shows the levels of incorporation of labelled precursor into lipid. In addition, DNA determinations were carried out on the cultured tissue fragments after lipid extraction in order to standardize the amounts of tissue and thus the rates of incorporation of precursors.

With ^{14}C-acetate as the precursor (Table VIIA) in the testosterone-treated epidermis, 2·55% of the available ^{14}C-acetate was incorporated into lipid, com-


(a)

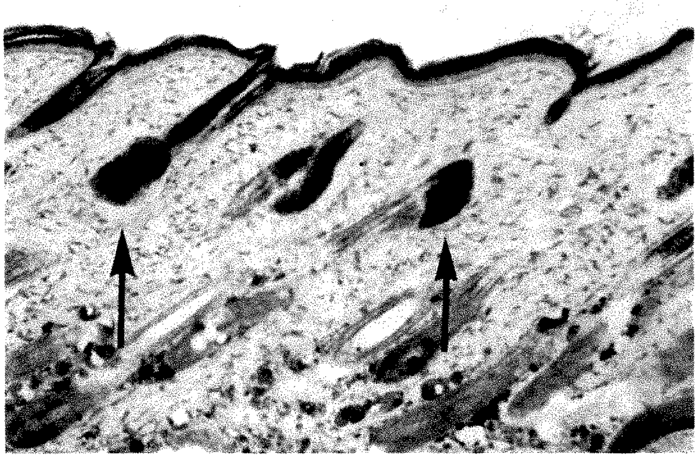

(b)

FIG. 5.—Effect of topically-administered methyl testosterone upon sebaceous glands. (a), control skin; (b), after methyl testosterone treatment (details of treatment given in text). Oil-red 0, ×81.

pared with only 1·52% in the control. However, although the sizes of epidermal fragments apparently taken were the same in both the treated and the control cultures, the DNA contents were also different. The *specific incorporation* into the two lipid samples, also shown in Table VIIA, indicate that there was no apparent difference in lipid synthesis between treated and control skin. In the dermal residues of the ^{14}C-acetate experiment, however, there were marked changes in both the proportion of acetate converted to lipid and in the specific incorporation into the lipid of the treated skin. The dermis of the testosterone-treated skin incorporated 7·32% of the ^{14}C-acetate into lipid, which represented a specific incorporation of 6097 dpm/μg DNA, whereas, in the control dermis, these values were respectively 1·92% and 1743 dpm/μg DNA.

The experiment was also performed using ^{14}C-glycerol as the substrate for lipogenesis. The results are shown in Table VIIB. Again, in the epidermis of the testosterone-treated skin there was no change compared with the control (1234 dpm/μg DNA compared with 1376 dpm/μg DNA), whereas, in the dermis, the testosterone-treated sample showed both higher proportions of glycerol incorporated into lipid and a higher specific incorporation. Thin-layer chromatographic analysis of the radioactive lipids of the control and testosterone-treated skin showed no marked changes in distribution, suggesting that overall lipid synthesis was stimulated in the dermis by the hormone.

DISCUSSION

There have been numerous papers describing the measurement of skin lipogenesis *in vitro*, utilizing the culture of tissues from various species in the presence of radioactive-labelled precursors of lipids (Nicolaides *et al.*, 1955; Vroman *et al.*, 1969; Wheatley *et al.*, 1970; Hsia *et al.*, 1970). In general, these studies have utilized short times of culture (up to 6 hours) and chemically defined media, such as Krebs-Ringer buffer. In the present work we have attempted to measure lipogenesis over extended periods, maintaining the tissues on a more complete culture medium, in the hope that viability and normal functioning of the skin would be better maintained. In previously reported work (Prottey and Ferguson, 1972) mouse auricular skin was cultured for up to 5 days, during which time the tissues appeared histologically quite normal. Incorporation of radioactivity into lipids was linear during 48 hours in culture. In preliminary experiments, rat skin was cultured in the medium which was successful for the mouse skin cultures, namely, Waymouth's medium 752/1 MB containing 10% calf serum, the skin being supported in the tissue culture cell described by Friend (1972). Although after 24 hours *in vitro* the rat skin exhibited reasonably normal morphology, after 48 hours there was evidence of gross loss of viability, with extensive cell vacuolation, necrosis, and pycnotic nuclei. Fig. 1 shows that, during a 24-hour culture of rat skin epidermis and dermis, the incorporation of both ^{14}C-acetate and ^{32}P-orthophosphate into structural and metabolic lipid components of the skin was reasonably linear. From these data we felt confident that the skin *in vitro* was functioning under conditions which were as near to normal as we could attain. The main differences in this study compared with other reports is that lipogenesis was measured over a 24-hour period, and that the culture medium was more complete, in that serum was added to a chemically defined medium. In other experiments with isolated epidermal cells *in vitro* we examined the effects of substituting different media (e.g. Eagle's medium; medium 199; and Waymouth's medium) together with different levels of serum. We found that all media were equivalent when the uptake of ^{14}C-acetate into lipids was the criterion, but medium 199 + 10% foetal calf serum and homologous rat serum gave the best maintenance of cell viability, measured histologically, during several days of culture (Prottey, unpublished data).

Systems for culturing tissues *in vitro* may not be wholly typical of the true state *in vivo*, since such tissues are contained in a closed environment, in contact with both complex culture media and end-products of metabolism, which may be toxic. Established lines of isolated cells in culture are even more atypical, since generally they are dedifferentiated and no longer carry out their specialized functions. Also, they have lost the specific orientation of their original environment so that any dependence upon, or interaction with, other cell types or regions of that environment is lost. In experiments concerning the biogenesis of lipids by tissues *in vitro* it has been reported that serum in the medium influences both the rate and the nature of lipids synthesized (reviewed by Rothblat, 1969). Cells in culture will tend to derive almost all of their lipids from the serum of the medium. Even if the ability to synthesize lipids is retained, the cells will preferentially rely upon exogenous lipids. Bailey (1964), using cultured L cells, found that ^{14}C-acetate incorporation into phospholipids was inhibited by 86% when serum was present. Avigan *et al.* (1970) similarly showed with skin fibroblasts that sterol synthesis was affected by the proteins and lipids of the serum, and Howard and Kritchevsky (1970), also using human diploid fibroblasts, showed that only 0·1% of the cellular free fatty acids were synthesized *de novo*, the remainder being derived from serum lipids. In the present study preliminary experiments were performed on rat skin epidermis (i.e. a 200 μm thick keratotome section) which was cultured in Waymouth's medium, both in the presence and absence of 10% calf serum. No differences in the rates of uptake of either ^{14}C-glycerol or ^{14}C-acetate into tissue lipids were seen. Also, the nature of the respective labelled lipid patterns were the same, whether serum was present or not. It was concluded that the " serum effect " was not significant in 24-hour cultures of freshly excised skin. In all subsequent cultures of skin 10% calf serum was added to the medium. Similar experiments were also performed using freshly isolated epidermal cells. Uptake of ^{14}C-labelled glycerol and ^{14}C-acetate was measured in medium 199, both in the presence and absence of serum (a mixture of 10% foetal calf serum and 10% homologous rat serum). Again, in both the serum-free and serum-supplemented media, no differences in the uptake of precursors into total lipids of the cells were seen, and the respective patterns of labelled lipids were identical. Thus, the presence of serum in the medium did not influence lipogenesis in the cultures of freshly isolated cells. The work reviewed by Rothblat (1969) was on established lines of cells which generally had been cultured through several passages, thereby allowing them to become acclimatized to serum lipids. Wilkinson (1970), however, has recently described the culture of freshly harvested human epidermal cells in a chemically defined medium and shown a higher incorporation of ^{14}C-acetate into cellular lipid in the absence of serum. If, however, the cells were first cultured in the presence of serum for 24 hours and then exposed to the radioactive precursor for a similar length of time, the situation was reversed, the cells in serum-supplemented medium incorporating about 10 times as much acetate as those deprived of serum. These results cannot be explained readily, however, and in our experience lipogenesis in cultures of freshly prepared rat epidermal cells is not influenced by the presence of foetal calf and rat sera.

In skin cultures of 24 hours duration the presence in the media of the antibiotics penicillin, streptomycin and mycostatin were always necessary to eliminate microbial contamination. Although there is a wealth of published information on the effects of various types of antibiotics upon protein—and nucleic acid metabolism (Miura, 1970), we are unaware of any data of their effects upon cutaneous lipogenesis. Many of the data described in this paper are quite similar to those of other authors (see Introduction). Indeed, we have recently measured lipogenesis in human skin under conditions quite similar to those described by Summerly and Woodbury (1971), except that we included the above mentioned antibiotics. Our results closely resembled those of these authors and would suggest that patterns of cutaneous lipids labelled *in vitro* from precursors such as ^{14}C-acetate are not influenced by antibiotics.

The lipids of skin may be classified into 3 groups, namely, those of the epidermis, which constitute structural components of the keratinizing epithelium; those produced by the sebaceous glands (which are holocrine glands associated with hair follicles, generally situated in the dermal matrix and connected with the skin surface by means of ducts which lead into the follicles); and those of the skin surface, which are spread as a thin film over the external surfaces of the skin and hair, and in combination with the stratum corneum. Lipids of the surface film are derived principally from sebaceous secretions, with a lesser contribution from the epidermis as by-products of keratinization. It was our intention to differentiate between lipogenesis in the epidermis and in the sebaceous glands, because such functionally different regions might possess different patterns of lipids, as is suggested by the results of others (Brooks *et al.*, 1963, 1966; Wheatley *et al.*, 1970; Hsia *et al.*, 1970). We have recently described differences in lipogenesis in mouse-ear skin, separated into dermis and epidermis by heat treatment after culture *in vitro* with specific lipid precursors (Prottey and Ferguson, 1972), but this technique is unsuitable for effecting a separation of tissues prior to culture, due to damage to Malpighian cells by the heat treatment. Trypsinization of whole skin, to separate epidermis from dermis, as reported by Brooks and co-workers is not suitable for rat skin, because generally we found the point of separation to be above the dermo-epidermal junction, with separated dermis containing most of the cells of the stratum basale. Similarly, the method of separating epidermis from dermis, using dilute salt solutions, which effect cleavage at the dermo-epidermal junction and swelling of the dermal collagen (Felsher, 1947), with the consequence that epidermis together with sebaceous glands may be removed intact from the underlying dermis, was not suitable for rat skin. Kellum (1966) used this procedure for preparing pure human epidermis plus sebaceous glands, as did Hsia *et al.* (1970) and Summerley and Woodbury (1971). For the relatively hairier rat skin, we found the method difficult to standardize. The Castroviejo Electrokeratotome has been successfully used for excising parallel sections of epidermis of constant thickness (Blank *et al.*, 1961). The technique of cutting specific skin regions was also used by Freinkel (1960), who described glucose oxidation in different regions of human skin *in vitro*. We found that, by using the 200 μm shim with 4–5 week old rats, the sections obtained ranged in thickness from 150–175 μm and contained the whole of the epidermis together with a small amount of the upper dermis, but none of the epidermal appendages. These remained in the residual dermis beneath the site of excision. The dermal residues were then excised and, when cultured with specific precursors, exhibited lipogenic activity. This activity is thought to be localized mainly in the sebaceous glands and possibly to a lesser extent in the hair follicle cells. Autoradiography of cultured mouse-ear skin (Prottey and Ferguson, 1972) has shown that, in the dermis, the sebaceous glands are very active sites of lipogenesis.

The data listed in Tables I–VII show that rat skin fractions in culture incorporated a variety of precursors into various lipids, and that differences were obvious between epidermis and dermis. Hsia *et al.* (1970), studying lipogenesis *in vitro* with fractions of human skin, reported that the " synthetic ability " of epidermis plus sebaceous glands was on average some 6 times that of the corresponding gland-free dermis, even although the dermis weighed about 10 times more than a corresponding area of epidermis. We cannot quote similar data, since we found tissue weight determinations to be too variable. However, in the experiment of Table VII, lipid radioactivities were standardized by reference to total DNA of the cultured skin, and it is seen with acetate as precursor that the epidermis had twice the synthetic ability of the dermis (containing sebaceous glands); with ^{14}C-glycerol as precursor the activities were about the same. We found in ^{14}C-acetate experiments that free sterols and phospholipids contained the majority of radioactivity in the epidermis, whereas, in the sebaceous glands, triglycerides and sterol/wax esters were more heavily labelled. These data are in accordance with the specific lipid requirements of the tissues. In the epidermis there is a high turnover rate of phospholipids and cholesterol which are the cell membrane components of a system actively undergoing cell division and keratin synthesis. The sebaceous glands, on the other hand, continuously produce sebum, which is generally enriched in triglycerides, wax esters and sterol esters, and, in some species, squalene (Nicolaides *et al.*, 1968). In the case of human skin (Hsia *et al.*, 1970), the same trends of lipid labelling from ^{14}C-acetate in epidermis and dermis were seen.

Table III shows the pattern of fatty acids labelled from ^{14}C-acetate in both epidermis and dermis. These results are somewhat equivocal, however. Although the distribution of radioactivity was within different fatty acids identified as rat skin constituents (Nikkari and Haahti, 1964; Nikkari, 1965), we never detected radioactivity in fatty acids of chain length longer than C18. The above-mentioned authors described fatty acids of chain length up to C26 in rat surface lipid, including straight chain and iso- and anteiso branched chain acids. We found also with cultured mouse-ear skin (Prottey and Ferguson, 1972) that fatty acids longer than C30 were not labelled, even although such acids are predominant in mouse-skin lipids (Carruthers, 1962). These failures of detection may have been due to the conditions of skin culture employed here, for Vroman *et al.* (1969) detected labelled behenic, lignoceric and cerotic acids in human skin cultured for only 6 hours with ^{14}C-acetate. In the present work gas-liquid radiochromatography was performed isothermally, with the consequence that very long chain fatty acids had large retention times. All chromatograms were run for up to 2 hours after the elution of C20 : 0, but no radioactivity was ever seen associated with very long chain fatty acids. Table III shows that traces of iso- and anteiso-branched fatty acids were labelled during culture. These acids have recently been shown to be synthesized by rat skin *in vivo* (Grigor *et al.*, 1970). In the lipid classes separated from rat skin we found that phospholipids and triglycerides generally contained labelled fatty acids which are characteristic of mammalian tissues in general (e.g. palmitic, palmitoleic, stearic and oleic acids), whereas the sterol/wax esters of both the epidermis and the sebaceous glands contained labelled fatty acids which are more uncommon, e.g. C15 : 0, anteiso-C15 : 0, iso-C16 : 0, C17 : 0, anteiso-C17 : 0 and iso-C18 : 0. We found no labelled octa-decadienoic acids, however, as reported by Wilkinson (1970) in human skin cultures.

The experiments using ^{14}C-mevalonic acid of Tables V and VI showed that sterol synthesis in both epidermis and dermis was qualitatively quite similar. Cholesterol was the principal labelled product during 24 hours in culture, and the build-up of radioactivity at shorter time intervals in an unidentified intermediate, squalene and lanosterol suggested that these compounds were intermediates. During 24 hours in culture more labelled squalene was found in the sebaceous gland-containing tissues compared with the epidermis. This may have been due to the specific synthesis of this lipid as a component of sebum, although Nicolaides *et al.* (1968) were unable to detect squalene in rat skin surface lipids. The labelling of sterol esters from ^{14}C-mevalonic acid was consistently low in both epidermis and dermis, although, in the experiments with ^{14}C-acetate, radioactivity was found in the acyl moieties (Tables II, III). Such data would suggest that the sterol moieties of sterol esters have very low rates of turnover compared with the free sterols, and may be derived, therefore, from the degeneration of cellular membranes of the keratinizing epidermis, or the maturing sebaceous cells. Wilson (1963) suggested that the conversion of mevalonic acid to cholesterol followed 2 distinct pathways in skin, in the epidermis the Bloch (1965) pathway being operative, whereas, in the sebaceous glands, the Kandutsch-Russell (1960) pathway, in which the intermediate sterols are thought to be esterified, was followed. Our results here do not clarify these ideas, since we did not identify the nature of the labelled sterol esters, but there are indications that sterol production in epidermis and sebaceous glands are very similar.

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THE EFFECT OF EICOSA 5:8:11:14 TETRAYNOIC ACID ON SKIN LIPID SYNTHESIS (^{14}C INCORPORATION) *IN VITRO*

ROY SUMMERLY, SHEILA WOODBURY AND H. J. YARDLEY

Summary.—The effect of 5 : 8 : 11 : 14 tetraynoic acid on the incorporation *in vitro* of ^{14}C acetate into the lipid fraction has been studied, using rat dorsum skin, rat tail sebaceous glands and human sebaceous glands.

An *in vitro* effect of the acid on skin lipid synthesis was demonstrated in rat dorsum skin, chiefly in the dermal samples which contained sebaceous glands and other epidermal appendages, consisting of a reduction in the total incorporation of ^{14}C acetate into the lipid classes and a reduction in the percentage incorporation into the wax ester/sterol ester fraction, with an associated increase in the percentage incorporation into the free fatty acid fraction. This *in vitro* effect was not obtained in the rat tail and human sebaceous gland yields.

The explanation for our inconsistent results using eicosa 5 : 8 : 11 : 14 tetraynoic acid, and those of other workers, is discussed.

In 1967 Strauss *et al.* reported that the oral administration of eicosa 5:8:11:14 tetraynoic acid to 8 male volunteers and 8 male acne patients resulted in an appreciable reduction in sebum excretion in all 16 subjects, with a coincident improvement in the skin lesions in the acne patients. Since that time 2 groups of British workers have investigated the effects and possible clinical applications of the compound when applied topically in acne patients (Burton and Shuster, 1970, 1972; Haroon *et al.*, 1970, 1972). Using a 2% ethanolic solution of eicosa 5:8:11:14 tetraynoic acid twice daily to the forehead skin, both groups reported a slight but significant reduction of the sebum excretion rate as compared to the control side, results which could not be confirmed when 20% propylene glycol was added to the ethanolic solution in an attempt to enhance absorption of the compound.

In view of the enigmatic results of these workers, we wish to report our own equally perplexing experiences. We have investigated the effect of eicosa 5:8:11:14 tetraynoic acid on the incorporation of ^{14}C acetate *in vitro* into the lipid fraction of rat dorsum skin, rat tail sebaceous glands, and isolated human sebaceous glands.

MATERIALS AND METHODS

Rat Dorsum Skin

In 5 separate experiments dorsum skin of a freshly-killed young adult male rat was used. The skin, with some subcutaneous fat, was removed from the animal and stretched

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taut on a wooden board. After shaving the skin, 8 circular areas of skin were delineated by means of a 10 mm biopsy punch—4 to serve as treated specimens and 4 as controls—selected from corresponding areas on each side of the mid-line. Using a Castroviejo keratotome (0·6 mm setting), lengths of skin were obtained from which the previously delineated circular areas could be lifted out. The specimens thus obtained had equal epidermal surface areas and uniform depth of dermis, with retention of all sebaceous glands without contamination by subcutaneous fat.

After culture (see below), the skin specimens were treated with 0·25% trypsin in buffered saline for 20 hours at 0°C, after which the epidermis could be peeled cleanly from the dermis (the sebaceous glands and other epidermal appendages remaining in the dermis). Dry weights were recorded and the components thereafter processed separately.

In the first series of experiments (Animals 1, 2 and 3) the first sample of eicosa 5:8:11:14 tetraynoic acid was used; in the second series, in one experiment (Animal 4) the second was used, and in another (Animal 5), the original sample.

Rat Tail Sebaceous Glands

In 4 separate experiments the proximal tail skin of a freshly killed young adult male rat was used. Eight thin rectangular slices of skin were cut by hand from each animal—4 as control specimens; 4 as treated specimens. After culture (see below), the pieces of skin were placed in 1 mol/l calcium chloride for 2–2½ hours at room temperature. At the end of this period the epidermis, with some of its appendages, could be peeled off, leaving many sebaceous glands which, with the aid of a Watson dissecting microscope, could be seen arranged in rows on the surface of the dermal component, from where they could be harvested by gentle pressure, using watchmaker's forceps. A pure yield of 50–100 sebaceous glands could be obtained in this way, and these were then transferred to previously weighed squares of aluminium foil and the dry weights recorded, using a Cahn Electrobalance Model M-10 (weight range of yields: 0·11–0·48 mg). Both samples of eicosa 5:8:11:14 tetraynoic acid were used.

Human Sebaceous Glands

Sebaceous glands were isolated after culture from upper back skin, obtained by drill biopsy (3 mm) under local anaesthesia in 2 patients (a male aged 58 who gave a history of acne vulgaris; a male aged 23 with cystic acne of the face) and from nasal skin of a male patient aged 73, obtained during decortication of rhinophyma under general anaesthesia. The first sample of eicosa 5:8:11:14 tetraynoic acid was used.

Culture medium

All skin specimens were cultured in a medium containing sodium 1-^{14}C acetate (38 μCi; 0·62 μmol) in 0·54 ml Krebs-Ringer bicarbonate, with added glucose. Eicosa 5:8:11:14 tetraynoic acid in 0·01 ml ethanol was added to half the culture bottles to produce a final concentration of the acid in the medium of 10^{-4} mol/l; ethanol 0·01 ml was added to the controls.

Skin Culture, Isolation of Human Sebaceous Glands, Extraction of Lipids, T.L.C. Techniques, Autoradiography and Counting Procedures. These were as described previously (Summerly and Woodbury, 1971).

RESULTS

Rat Dorsum Skin

The results in Tables IA and B of experiments in which both samples of eicosa 5 : 8 : 11 : 14 tetraynoic acid were used, demonstrate a significant reduction in the total incorporation of ^{14}C acetate into the lipid fraction of epidermis and dermis

TABLE I.—*Pattern of Incorporation of ^{14}C-acetate into the Lipid Fraction (Expressed as % of total incorporation present in each spot)*

A. *Rat Dorsum Skin* (3 *male animals*, 11 *treated observations*, 11 *control observations*)

Tetraynoic acid Sample 1

Epidermis	Origin*	Diglyceride free sterol	Free fatty acid	Triglyceride	Spot 6	Wax esters†	Squalene	Total incpn (disint/sec/mg)
Treated	45·3±1·3	28·1±0·8	11·6±1·0	7·2±0·5	1·7±0·2	4·5±0·3	1·7±0·2	52·6±5·5
Controls	53·7±2·1	20·4±1·0	8·2±1·0	9·8±0·5	1·4±0·1	5·8±0·7	0·9±0·1	82·3±9·3
Probability								$0·02>P>0·01$
Dermis								
Treated	25·5±1·4	10·9±0·9	18·4±0·5	13·6±0·6	5·1±0·6	16·6±0·7	9·9±1·0	23·6±3·3
Controls	26·7±1·0	6·5±0·4	9·3±0·6	18·3±0·8	3·5±0·4	30·4±1·0	5·3±0·5	36·0±5·2
Probability			$P<0·001$			$P<0·001$	$P<0·001$	$0·10>P>0·05$

B. *Rat Dorsum Skin* (2 *male animals*, 8 *treated observations*, 8 *control observations*)

Tetraynoic acid Samples 1 and 2

Epidermis								
Treated	34·6±0·9	27·6±0·9	10·3±0·8	14·0±0·7	3·5±0·3	8·8±0·9	1·2±0·2	60·0±3·1
Controls	43·6±1·0	20·9±1·2	9·6±0·4	11·9±0·8	3·5±0·4	9·5±1·3	1·0±0·1	93·1±7·5
Probability								$0·005>P>0·001$
Dermis								
Treated	19·4±1·1	6·1±0·4	6·9±0·3	17·0±0·6	7·1±0·5	34·0±1·1	9·5±1·3	86·6±13·6
Controls	21·3±0·8	4·4±0·3	6·0±0·2	18·1±0·8	5·3±0·5	39·3±1·2	5·6±0·5	159·5±12·3
Probability						$0·02>P<0·01$	$0·05>P>0·02$	$0·005>P>0·001$

C. *Rat Tail Sebaceous Glands* (4 *male animals*; 16 *treated observations*; 16 *control observations*)

Tetraynoic acid Samples 1 and 2

Treated	17·3±0·9	3·2±0·2	8·3±0·3	18·2±0·8	1·6±0·1	23·2±0·7	28·1±1·3	70·7±6·4 ($\times 10^3$)
Controls	16·5±1·3	2·9±0·1	6·5±0·4	17·2±1·0	1·8±0·2	24·1±1·0	30·9±2·1	74·8±8·9 ($\times 10^3$)

D. *Human Sebaceous Glands* (3 *male patients*; 4 *treated observations*; 3 *control observations*)

Tetraynoic acid Sample 1

Treated	8·2	3·5	2·2	44·8	1·8	23·5	16·0	177 ($\times 10^2$)
Controls	8·0	3·2	1·9	42·9	1·1	24·4	18·5	232 ($\times 10^3$)

Total Incorporation—2 observations only.

KEY: * polar lipid including phospholipid.
† includes sterol (cholesterol) esters.
Spot 6 = probably polar wax ester (see Summerly and Woodbury, 1971).
Results expressed as means ± S.E.M.

as compared to the controls; and an alteration in the pattern of incorporation, which was most obvious in the dermal specimens in the first series of experiments, consisting of a mean reduction by 45% of the percentage incorporation into the wax ester/sterol ester spot, with a mean increased percentage incorporation of 98% into the free fatty acid fraction (Table IA). A less striking but significant reduction of 13·5% in the mean percentage incorporation into the wax ester-sterol ester fraction was also demonstrated in the dermis in the second series (Table IB).

Rat Tail Sebaceous Glands

Table IC shows the lack of effect of eicosa 5:8:11:14 tetraynoic acid 10^{-4} mol/l *in vitro* (both samples) on the total incorporation and pattern of incorporation of ^{14}C acetate in the lipid fraction measured in pure yields of rat tail sebaceous glands.

Human Sebaceous Glands

No effect of eicosa 5:8:11:14 tetraynoic acid 10^{-4} mol/l *in vitro* (Sample 1) was observed on the pattern of incorporation of ^{14}C acetate into the lipid classes of isolated human sebaceous glands (4 observations). In Patient 3 (2 observations only), the total incorporation into the lipid fraction expressed per mg dry weight was reduced in the treated samples (Table ID).

In Patients 1 and 2 (mean of 2 observations) no difference was detected in the pattern of incorporation of ^{14}C acetate into the overlying appendage-freed epidermis in the treated samples as compared to the controls (percentage incorporation into each lipid class reading from origin spot → squalene spot: *Treated:* 32·9, 7·7, 6·2, 12·2, 22·7, 1·6, 10·0, 6·7; *Controls:* 33·3, 9·0, 6·8, 14·4, 20·5, 1·6, 8·1, 5·8). Unfortunately no values were obtained for the total incorporations, expressed on a dry weight basis.

DISCUSSION

The oral administration of eicosa 5 : 8 : 11 : 14 tetraynoic acid, an effective premevalonate inhibitor of cholesterol synthesis (unpublished work, cited by Strauss *et al.*, 1967), to young male acne patients and control volunteers for periods ranging from 7–19 weeks resulted in a marked reduction in sebum excretion (mean reduction both groups 44%; acne group 52%; control volunteers 32%), with coincident improvement in the skin lesions in the acne patients (Strauss *et al.*, 1967). This observed inhibition of lipid biosynthesis by the sebaceous glands was considered by Ahern and Downing (1970) to be due to reduced adrenal steroid production, the consequence of depressed prostaglandin synthesis, the eicosa 5:8:11:14 tetraynoic acid having been shown to block conversion of its ethylene analogue, arachidonic acid, to prostaglandin E_2 by sheep seminal vesicle tissue. The inhibition of the conversion of 2 other polyethylenic fatty acids (linoleic, linolenic) to hydroxy-acids was also demonstrated by these workers. Ahern and Downing (1970) postulated that eicosa 5:8:11:14 tetraynoic acid is converted to an allene by prostaglandin synthetase, and that this allene combines irreversibly with histidine groups at the active sites of the enzyme.

That the effect of eicosa 5:8:11:14 tetraynoic acid might be a direct one on the lipid-synthesizing components of skin, rather than a systemic effect mediated through the adrenal cortex, was suggested by the preliminary studies of

Burton and Shuster (1970) and Haroon *et al.* (1970). These workers showed that the twice-daily application for 6–8 weeks of 2% eicosa 5:8:11:14 tetraynoic acid in ethanol to one side of the forehead resulted in a small but significant reduction in the sebum excretion in all 16 subjects and, in one patient, improvement of acne on the treated side. These results could not be reproduced in later trials by these workers when, to improve percutaneous absorption, 20% propylene glycol was added to the ethanolic solution of the acid (Burton and Shuster, 1972; Haroon *et al.*, 1972).

Utilizing rat dorsum skin (Tables IA and B), we have demonstrated a direct and early *in vitro* effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid, shown most strikingly in the dermal samples of the first 3 animals used (Table IA). Considerable variation in the *in vitro* effect occurred in these experiments, the alteration in pattern of incorporation being less obvious in the second series (Table IB) when the original sample of tetraynoic acid (after storage) and a second sample were used.

The altered pattern of incorporation we have demonstrated cannot be entirely accounted for on the basis of a pre-squalene inhibition of cholesterol with less available for esterification, as it seems improbable that this would result in as much as a 45% reduction in the percentage incorporation of ^{14}C acetate (or a 64% reduction in the *absolute* amount incorporated) into the wax ester/cholesterol ester spot (Table IB). In the light of the findings of Ahern and Downing (1970), it seems possible that some interference in wax ester synthesis might also be operating, the increased percentage incorporation (98%) in the free fatty acid fraction (Table IB) representing retention in the fatty acid " pools " from which triglycerides, sterol esters and wax esters are normally synthesized.

Our further experiments, from which it was hoped, by studying the ^{14}C incorporation in pure yields of rat and human sebaceous glands, to confirm the effect of eicosa 5:8:11:14 tetraynoic acid on cholesterol ester and/or wax ester synthesis, proved disappointing, as no alteration in the pattern of incorporation was detected. In the rat sebaceous glands no reduction in the total incorporation occurred (Table IC), while only a small reduction in the total incorporation in the human sebaceous glands was noted; but the number of observations (2) precludes any firm conclusion (Table ID).

Explanations for the *in vitro* effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid being initially so convincing but eventually unsustained must take into account varying species and organ susceptibilities or, as seems most likely, instability of the acid. Holgate (1970) has emphasized the instability of the compound in an atmosphere containing oxygen or at an inimicable pH—such as would obtain in the *in vitro* system used—when precipitation of the free acid out of solution might have occurred.

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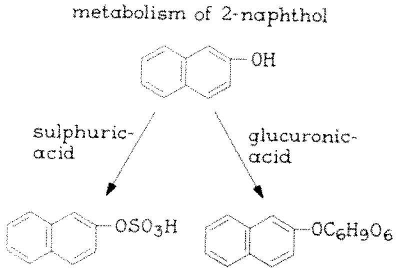
PERCUTANEOUS ABSORPTION AND DISTRIBUTION OF 2-NAPHTHOL IN MAN

H. G. W. M. HEMELS

Summary.—A procedure is described for determining unchanged and conjugated 2-naphthol in plasma and urine. The method, based on gas chromatography is rapid and has a limit of detection of approximately 2 $\mu g/m/l^{-1}$. Twenty-four hours urine excretion data of 2-naphthol, after therapeutic application of a 2-naphthol-containing ointment on the skin of patients, are reported. Plasma levels of 2-naphthol and conjugated 2-naphthol during and after topical treatment with a 2-naphthol peeling paste were measured in 4 patients. Plasma levels of 2-naphthol were significantly lower than the plasma levels of conjugated 2-naphthol. The cutaneous barriers were found to be easily traversed by 2-naphthol. The relevance of the data for clinical practice is discussed.

2-NAPHTHOL (β-naphthol) is a powerful dermatotherapeutic agent, which has been extensively used since its introduction by Ludwig and Kaposi in 1881. Before the advent of modern antihelminthics, 2-naphthol was also used as a vermicide, for which purpose it proved to have little therapeutic value because of its toxicity (Smillie, 1920). The effectiveness of 2-naphthol in peeling pastes for the treatment of persistent acne vulgaris has kept this drug in the current pharmacopoeias, although with restrictions as to its dosage (Mali, 1970; Percival, 1967; Volk and Winter, 1936; Powell, 1970). In contrast to the large amount of data about side effects (Albignente, 1968; Anon., 1922; Gleason *et al.*, 1969; Clarke, 1969; Osol and Farrar, 1947), there are relatively few reports at the biochemical and pharmacological levels (Powell, 1970; Harkness and Beveridge, 1966). 2-naphthol, administered to animals, is excreted as a conjugate of glucuronic acid, as free 2-naphthol and, to a minor extent, as ethereal sulphate (Lesnik and Nencki, 1886; Berenbom and Young, 1951; Boyland and Wiltshire, 1953; Corner and Young, 1954, 1955). This paper will deal with the development of a highly



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sensitive and reasonably rapid analytical method for the determination of 2-naphthol and its conjugates in blood and urine. This method has been used to determine the 24 hours urine excretion pattern of patients topically treated with a 2-naphthol-containing ointment. Plasma levels of 2-naphthol and conjugated 2-naphthol were measured during one day of treatment.

MATERIALS AND METHODS

Reagents.—All reagents used were analytical grade (Merck).

Apparatus.—A Hewlett Packard model F and M 402 high efficiency gas chromatograph with a flame ionization detector was used. It was coupled to a Hewlett Packard Moseley recorder, model 7127 A. The gas chromatograph was operated under the following conditions: 180 cm × 0·3 cm i.d. u-shaped glass column packed with 3·8% UCW 98 on 80/100 mesh Chromosorb W (dimethyl-dichlorosilane, high performance); nitrogen gas with a flow rate of 50 ml/min; hydrogen flow 50 ml/min; air flow 600 ml/min; column temperature 160°C; injection port, flash heater and detector temperature 190°C.

Extraction procedures.—(a) The unchanged 2-naphthol (free). Five ml urine or 2·0 ml plasma was acidified with 1 ml 1N hydrochloric acid and immediately extracted 3 times with 15 ml peroxide-free ether in a 40 ml glass-stoppered tube by shaking on an automatic shaker for 10 min. The ether extracts were dried with anhydrous magnesium sulphate and evaporated to dryness in a 25°C waterbath under nitrogen.

(b) The unchanged and conjugated 2-naphthol (total). Five ml urine or 2·0 ml plasma was acidified with 1 ml concentrated hydrochloric acid and the glass-stoppered tubes immersed in a waterbath at 70°C for 4 hours (Bakke and Scheline, 1969). The 2-naphthol was extracted as described above.

Determination.—The residues from the ether extraction were dissolved in ethyl acetate (0·10 ml–5·00 ml, dependent on concentration) and 3 μl of the solution was injected into the gas chromatograph. Each sample was run 3 times, and an average of the 3 determinations was taken. The sensitivity of the instrument was checked each hour with 2-naphthol standard solutions at known concentrations. Anthracene can be used as an internal standard.

Patients and treatment.—The subjects studied were patients aged from 18–22 years

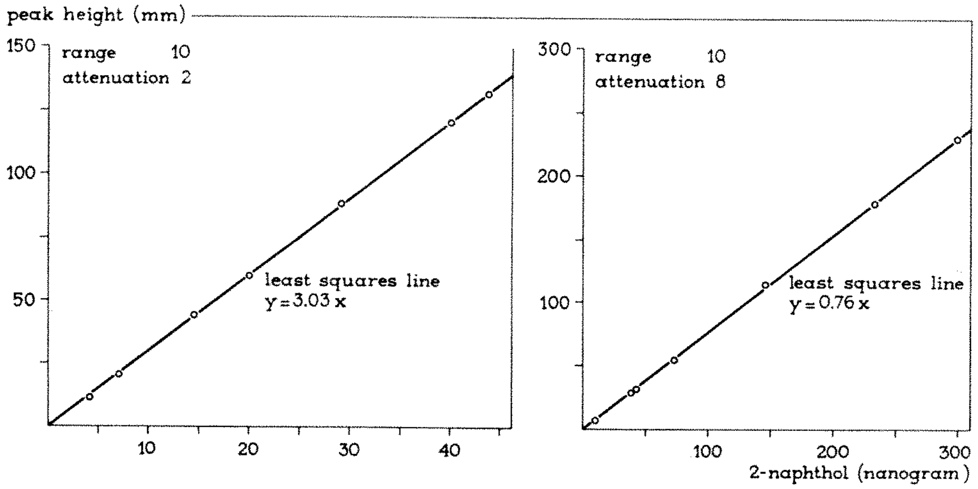

FIG. 1.—Standard calibration curves of the peak height in mm against the amount of 2-naphthol in ng.

under treatment for gross acne vulgaris, involving face, neck and trunk in various degrees. The subjects were free of demonstrable hepatic and renal disease. The so-called peeling paste contained 20% 2-naphthol, 20% soft soap, 10% precipitated sulphur and 50% soft paraffin. The paste was applied daily to about 5% of the body surface with a spatula, covered with bandages and left on the skin for approximately 7 hours. The applications were repeated until marked desquamation with inflammation resulted in removal of comedones and suppression of the acne.

Urine and blood samples.—(a) Twenty-four hours urine was collected during the hospitalization of 10 subjects treated with 7·5 g paste.

(b) Blood and urine samples of 4 subjects were taken on one or more days during the treatment period. Venous blood samples were collected in heparinized tubes at the following intervals from the time of application of the peeling paste: 0, 1, 3, 8, 12 and 24 hours. Urine was collected in such a way as to coincide with the time of blood sampling. The free plasma and urine 2-naphthol was extracted on the day of collection. The samples were stored in a refrigerator until used.

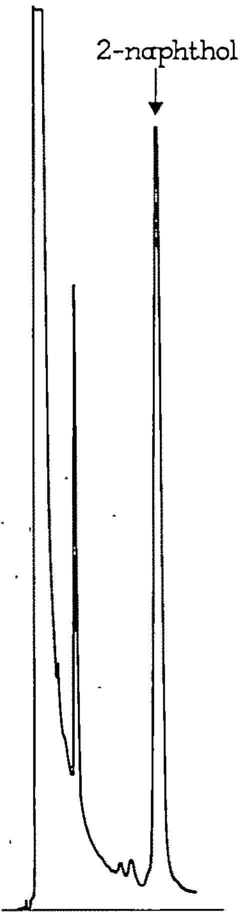

FIG. 2.—Gas liquid chromatogram of an extract from urine of a patient treated with a 2-naphthol peeling paste.

RESULTS

Calibration curves and recovery data

The analyses of urine and plasma of control subjects show no peaks that interfere with the 2-naphthol determinations. Calibration curves for 2-naphthol determinations are shown in Fig. 1. A gas chromatogram of an extract from patient's urine is shown in Fig. 2. In order to determine the recovery of 2-naphthol in the extraction procedure, known amounts of 2-naphthol were added to plasma and urine samples of control subjects. Tables I and II summarize the recovery data when the drug was extracted from urine and plasma respectively. The percentage of recovery was considered satisfactory.

(a) *Twenty-four hours urinary excretion data.*—The mean 24 hours urinary excretion data of 2-naphthol and conjugated 2-naphthol from 9 patients during the period of treatment (18–35 days) with 7·5 g 2-naphthol peeling paste are summarized in Table III. Subject 10 was treated with a very small amount of paste. The proportion of the dose applied daily (1·5 g 2-naphthol) to the amount which was recovered in the urine of that day had a mean value of 5% (over 250 applications), with a maximum of approximately 41%. The average percentages of free 2-naphthol present in the average amount of total excreted drug varied considerably between extremes of 13% and 64%, with a mean value of about 37%. The 24 hours urinary excretion data during 24 days of treatment of Subject 5 are shown in Fig. 3. Treatment of the back of the patients resulted in the highest amounts being found in the urine. In Fig. 3 the open circles in the range 3·5 mg %–7·5 mg % (50 mg–146 mg) all represent values during the application of the 2-naphthol ointment to the back of the subject.

TABLE I.—*Recovery Data of 2-naphthol Added to 5 ml Urine Samples*

Added amount 2-naphthol to 5·0 ml urine		Recovery data	
μg	P.p.m.	μg	%
1·0	0·2	1·05	107
1·0	0·2	1·09	—
10·0	2·0	10·2	101·5
10·0	2·0	10·1	—
20·0	4·0	19·7	99·5
20·0	4·0	20·1	—
50·0	10·0	50·1	99·6
50·0	10·0	49·5	—

TABLE II.—*Recovery Data of 2-naphthol Added to 2 ml Plasma Samples*

Added amount 2-naphthol to 2·0 ml plasma		Recovery data	
μg	P.p.m.	μg	%
1·0	0·5	1·06	108
1·0	0·5	1·09	—
10	5·0	9·4	95
10	5·0	9·5	—

(b) *Plasma and urine analyses.*—From Fig. 4 it can be seen that the plasma levels of free 2-naphthol were different for the 7 test days of 3 subjects. A slight rise in the plasma level was observed, which reached a peak level 12 hours after

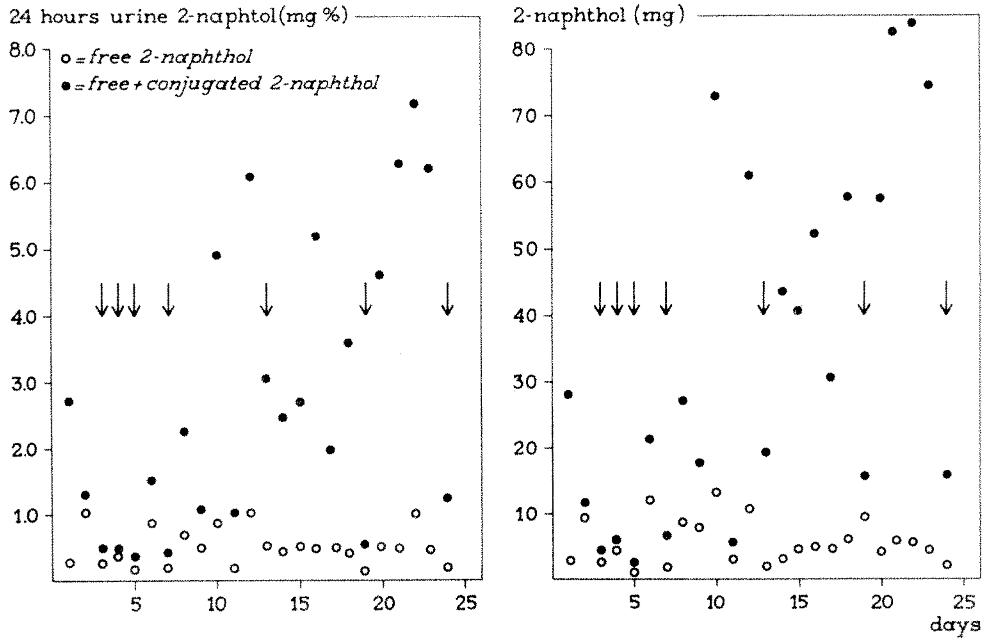

FIG. 3.—Urinary 2-naphthol and total (free + conjugated) 2-naphthol measured in 24 hour aliquots, during the time of hospitalization of Patient 5 treated with 7·5 g 2-naphthol peeling paste. Arrows indicate days of no treatment.

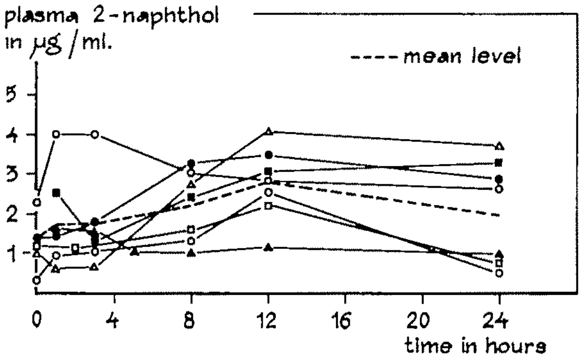

FIG. 4.—Plasma levels of 2-naphthol during and after 7 hours exposure to a 20% 2-naphthol peeling paste in 3 patients with acne. White circles: H,28/12, 10 g paste applied to the face; white circles with point: H,6/1, 10 g face; black dots: H,13/1, 10 g back; white squares: B,6/1, 5 g face; black squares: B,28/12, 5 g face; white triangles: L,26/1, 10 g face; black triangles: L,16/2, 10 g face.

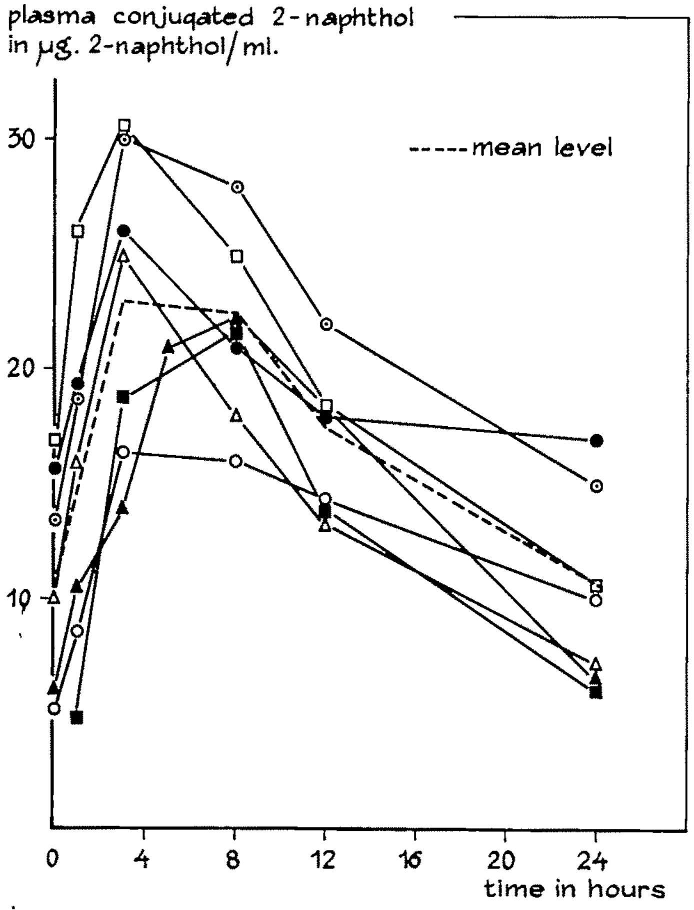

FIG. 5.—Plasma levels of 2-naphthol conjugates during and after 7 hours exposure to a 20% 2-naphthol peeling paste in 3 patients with acne. For explanation of symbols see Fig. 4.

TABLE III.—*Twenty-four Hours Urine Excretion Data of 10 Subjects During Treatment with a 2-naphthol Peeling Paste. With the Exception of Subject 10, 7·5 g Peeling Paste Was Used Each Day of Treatment.*

	Urinary excretion data free 2-naphthol				Urinary excretion data total 2-naphthol				
Subject	Mean mg%	Range mg%	Mean mg	Range mg	Mean mg%	Range mg%	Mean mg	Range mg	Free amount %
1	2·1	0·4–4·7	24·6	8·0– 52·5	3·8	0·6– 9·7	50·6	12·1–145·5	55
2	2·7	0·8–4·8	35·7	11·6– 63·8	4·6	1·7– 7·3	57·9	24·3–146·1	59
3	1·6	0·2–4·0	17·2	3·2– 51·2	5·1	3·5–10·1	53·5	2·1–141·2	31
4	1·1	0·2–1·2	17·8	2·6– 60·8	2·0	0·8– 4·9	32·4	9·1– 76·3	55
5	0·6	0·2–1·1	6·4	1·8– 13·5	3·5	1·0–17·9	39·9	6·0–134·3	17
6	2·3	0·6–5·2	30·8	8·4– 58·1	7·7	2·5–22·9	95·3	36·4–309·3	30
7	1·5	0·2–3·9	22·5	2·0– 70·1	4·7	1·1–13·3	70·7	11·6–143·7	32
8	3·2	0·8–6·7	66·2	10·5–187·6	5·0	0·9– 9·9	102·1	13·9–277·8	64
9	1·3	0·5–2·3	25·8	11·0– 43·8	9·1	1·4–21·6	197·8	28·6–612·5	14
10	0·04	– –0·3	0·5	– – 5·4	0·3	– – 5·4	3·6	– – 8·2	13

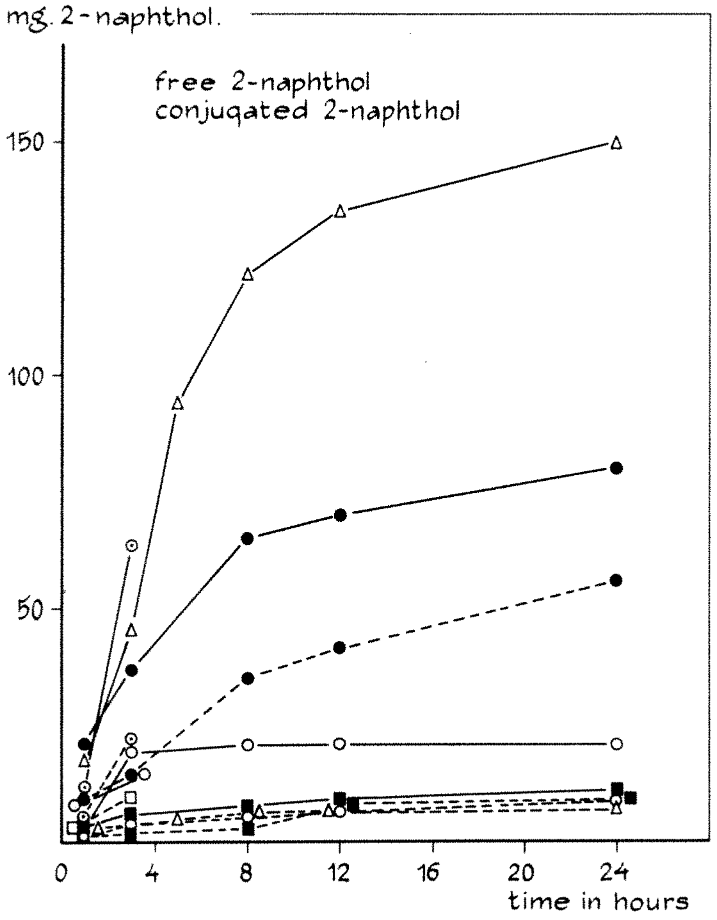

FIG. 6.—Cumulative excretion of 2-naphthol (broken lines) and conjugated 2-naphthol in the same patients. See Fig. 4.

application of the paste. The highest value was approximate 4 $\mu g/ml^{-1}$. Two days after the last application the free 2-naphthol began to disappear from the blood. The plasma level of free 2-naphthol was far less than the plasma level of conjugated 2-naphthol. The plasma levels of conjugated 2-naphthol evaluated for the same 7 test days are shown in Fig. 5. In 5 tests the plasma levels of conjugated 2-naphthol reached peaks 3 hours after application of the paste. However, in the other 2 tests, peak values were found 8 hours after the application. The highest mean peak level, noted 3 and 8 hours after application of the paste, were 23·0 $\mu g/ml^{-1}$ and 21·7 $\mu g/ml^{-1}$ respectively. Thereafter the levels decreased: 24 hours after application of the paste the mean value was 10·4 $\mu g/ml^{-1}$. The amount of conjugated 2-naphthol in the plasma of 2 subjects 48 hours after the last application was 3·6 $\mu g/ml^{-1}$ and 3·2 $\mu g/ml^{-1}$ respectively. These values decreased further to 1·1 $\mu g/ml^{-1}$ and 0·5 $\mu g/ml^{-1}$ the next day. The elimination rate constants of the conjugated 2-naphthol were obtained by means of the graphical analyses of the plasma concentrations against time, using semilogarithmic graph paper. The elimination coefficient (k'_{el}) and the apparent biological half life ($t'_{50\%} = \ln 2/k'_{el}$) calculated for the conjugated 2-naphthol of 4 subjects are summarized in Table IV. The cumulative excretion data are shown in Fig. 6.

TABLE IV.—*Elimination Constant, k'_{el} and Apparent Biological Half Life, $t'_{50\%}$ of Conjugated 2-naphthol in the Different Tests on 4 Subjects. "G paste" is the Amount of 2-naphthol Paste Applied on the Day of the Experiment; --- Indicates One Day After Cessation of Therapy.*

Subject	Date	G paste	Application area	k'_{el} in h^{-1}	$t'_{50\%}$ in h
H	28/12	10	Face	0·031	22·4
H	6/1	10	Face	0 039	17·8
H	13/1	10	Back	0·021	33·0
H	26/1	---	---	0·050	14·0
B	28/12	5	Face	0·079	8·8
B	6/1	5	Face	0·066	10·5
B	13/1	---	---	0·073	9·5
L	26/1	10	Face	0·063	11·0
L	16/2	10	Face	0 086	8·1
J	1/3	---	---	0·059	11·8

These urinary excretion data showed different values for each test day. The highest urinary excretion rates were observed during the first hours after application of the paste. Unfortunately, some urine samples were lost.

DISCUSSION

(a) *Twenty-fours hours urinary excretion data.*—The 24 hours urinary excretion data show that there is an average recovery of about 5% of the dose applied to about 5% of the body surface area. Harkness and Beveridge (1966) reported the recovery in urine of about 10% of the total amount of 2-naphthol applied in a peeling paste to about 20% of the surface area of the subject. It is difficult to compare these results because the pastes used differed in their composition (concentration, vehicle). Application of the paste on the back of the patients resulted in the highest urine values. Here the application area may have been greater because of squeezing and spreading under the dressings. The amount of free 2-naphthol excreted in the urine as a percentage of the total amount excreted (mean value 37%) is similar to that in rats after subcutaneous injection of

2-naphthol; it was found that 33–44% of the total amount of 2-naphthol excreted in the urine was not conjugated (Berenbom and Young, 1951). The great variation in the percentages of free 2-naphthol compared to the amount of total 2-naphthol excreted could be due to differences in metabolic capacity of the subjects and/or of variations in the pH value of the urines; 2-naphthol is a weak acid. The passive tubular reabsorption of the unchanged drug depends on the amount of lipid-soluble, non-ionized form present, which in turn is dependent on the pH value of the urine. The maximum concentration found in the urine was 67 μg/ml^{-1}.

(b) *Plasma and urine analyses.*—The clinical effect of the application of a 2-naphthol peeling paste (Powell, 1970) and the toxicological aspects of this treatment have been considered to result from the penetration of this drug through the skin. However, there has never been any reliable report on plasma levels of 2-naphthol. If we suspect an intoxication due to 2-naphthol, we have to realize that a 2-naphthol plasma level will give insufficient information about the degree of exposure of the subject to this compound. From Fig. 4 and Fig. 5 it can be seen that, when 2-naphthol enters the circulation, the amount of free 2-naphthol remains nearly constant at a low level. The ratio of free drug to conjugated drug in the plasma appeared not to be constant. The appearance in the plasma of the conjugated 2-naphthol is apparently capacity-limited; it follows zero order kinetics. The 2-naphthol is readily absorbed. Rapid conjugation and renal excretion of the unchanged drug seems to be the principal mechanism of elimination. The long-lasting plasma level of 2-naphthol could be caused by absorption from skin- and tissue-depôts, tubular reabsorption and enterohepatic circulation of the drug (Wagner, 1971; Riegelman, 1971; Smith, 1966; Williams *et al.*, 1965). In 4 subjects the apparent biological half life ($t'_{50\%}$) of conjugated 2-naphthol varied between extremes of 8 hours and 33 hours. Accumulation of 2-naphthol in man was reported by Lesnik and Nencki as early as 1886. The present absorption studies of topically-applied 2-naphthol in a so-called peeling paste showed that there was a considerable percutaneous absorption of 2-naphthol. The cutaneous barriers could be easily traversed by the drug. The entire stratum corneum has to be considered as the rate-limiting barrier. Treatment of the skin with a peeling paste, however, may change the conditions in such a way that percutaneous absorption occurs readily (Blank and Scheuplein, 1969; Montagna and Lobitz, 1964). A potential hazard may be created, particularly in patients with widespread acne who are treated during a prolonged period on a relatively large fraction of the body surface. Therefore the paste should be applied only to limited areas (150 cm^2) of patients with normal glucuronidating systems. The patient should be followed closely during therapy, with careful clinical and laboratory observations. The paste should not be left on the skin for more than one hour. Treatment carried out twice a day for a short time is preferable to one long exposure. A high output of urine—preferably alkaline—must be ensured. It is obvious that pregnancy is a contraindication to such therapy.

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STRUCTURAL AND FUNCTIONAL ABNORMALITIES OF THE LIVER IN PSORIASIS BEFORE AND DURING METHOTREXATE THERAPY

J. ALMEYDA, D. BARNARDO, H. BAKER, G. M. LEVENE
AND J. W. LANDELLS

Summary.—The treatment of psoriasis with methotrexate carries a low but definite risk of producing histological abnormalities in the liver. Forty-two patients treated for 3 to 80 months were found to have more histological abnormalities than 25 untreated patients with equally severe psoriasis. Of the treated patients, 3 had cirrhosis, all of whom were heavy drinkers, and 12 had fibrosis. None of the untreated patients had cirrhosis, but 4 had fibrosis. Only the cirrhotic patients showed clinical evidence of hepatic inpairment.

The incidence of histological abnormality increased with increasing cumulative dosage of methotrexate, but patients on weekly therapy had less abnormality than those on daily oral therapy.

Fourteen of the 25 untreated patients were given the drug subsequently, once weekly for a mean period of 23 months. Very few additional histological abnormalities have developed.

In patients with socially and physically crippling psoriasis where methotrexate would be used, the risk of significant hepatic damage is low enough to be acceptable, providing supervision is thorough.

FOLIC acid antagonists have been used in the treatment of severe psoriasis for almost 20 years. Liver function tests are known to become temporarily abnormal during therapy (Ryan *et al.*, 1964), but only recently has overt liver disease been attributed to methotrexate (Coe *et al.*, 1968; Muller *et al.*, 1969; Epstein and Croft, 1969). These reports have to be assessed in relation to hepatic abnormalities found on liver biopsy in psoriatic patients never treated with methotrexate (Berge *et al.*, 1970).

This paper reports our findings in 67 patients with severe psoriasis from whom liver biopsies were obtained before, during, or after methotrexate therapy.

PATIENTS AND METHODS

Sixty-seven patients were studied (39 males, 28 females). Twenty-five were investigated before methotrexate was given, and these formed a " control " group for comparison with the other 42 patients who were studied during or after methotrexate therapy. The former will be referred to as the " untreated " patients and the latter as the " treated " ones.

The treated and untreated groups were comparable in respect of age and duration of psoriasis. The age range for the treated group was 21–77 years (mean 55 years),

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and for the untreated group was 16–77 years (mean 48 years). The duration of psoriasis was 8–47 years (mean 17 years) and 1–35 years (mean 18 years) respectively. There was no significant difference between these values or their distribution. All patients had severe psoriasis, but 3 categories were distinguished: widespread intractable discoid psoriasis (WD), generalized exfoliative (erythrodermic) psoriasis (E), and generalized pustular psoriasis (GP) (Fig. 1).

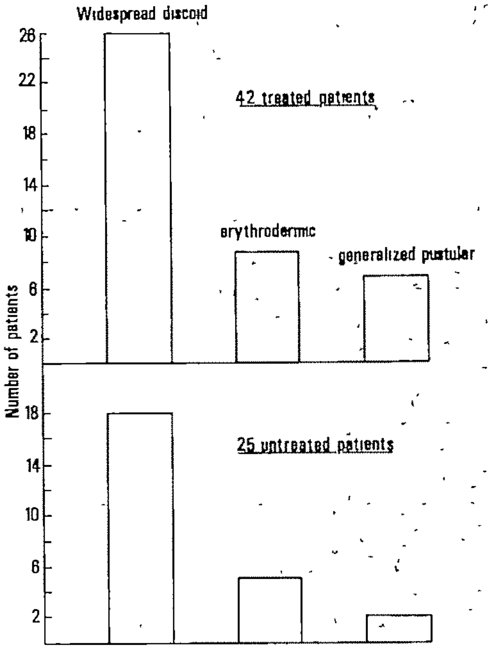

Fig. 1.—Categories of psoriasis.

A detailed alcohol history was obtained. Patients were graded as light (or nil), moderate, or heavy drinkers. A heavy alcohol intake was defined as being a regular average daily intake of more than 3·5 l of beer or the equivalent in spirits. The incidence of heavy drinking is shown in Table I. There was no statistically significant difference in the incidence of heavy drinking in the 2 groups. Most patients had been exposed to tar, dithranol or corticosteroids topically; a few had received oral corticosteroids or inorganic arsenic in the past. It was impossible to assess the effects on the liver of these agents. A number of patients had received paracetamol, indomethacin, salicylates and tranquillizers; there was no correlation between exposure to these drugs and our findings. One patient gave a history of jaundice attributed to hepatitis,

Table I.—*Ethanol Consumption in Treated and Untreated Patients*

		Ethanol Consumption		
	Total patients	Heavy	Moderate light or nil	Not known
Treated	42	7	32	3
Untreated	25	2	21	2

and 3 had a history of cholelithiasis (all had normal liver biopsies). Otherwise there was no past history of liver disease in either the treated or untreated patients.

Three methotrexate dosage schedules were used—" daily oral " (2·5 mg taken orally on 4 or 5 days each week, or daily on alternate weeks) (11 patients); " weekly oral " (12·5–25 mg taken as a single oral dose once a week) (18 patients); and " weekly parenteral " (20–40 mg given intramuscularly or intravenously at weekly or greater intervals) (13 patients). Routine " liver function tests " included serum bilirubin, serum amino-transferase and alkaline phosphatase (without isoenzyme analysis) in all patients. The alkaline phosphatase was ignored in one patient known to have Paget's disease of the bone. Bromsulphthalein (BSP) retention was estimated in all but 4, the upper limit of normal being 6% BSP retention at 45 min. Biopsy specimens were obtained, using either the Menghini or Vim Silverman needles, and were fixed immediately in formol saline. All sections were stained with H. and E., iron, reticulin and van Giesen stains, and were examined by one of us (JWL) without reference to the clinical or biochemical features. Abnormalities were grouped into 4 categories: accumulation of fat in the liver cells, appearance of inflammatory cells in the portal tracts, development of collagen and reticulin fibres in excess of normal (fibrosis), and fibrosis with accompanying nodular regeneration (cirrhosis). The first 3 histological changes were assessed on a 4-point scale, 0–3; fat and inflammatory cell changes were grouped together as " non-specific " changes; histological changes in the cirrhotic patients were not sub-divided (Table II).

TABLE II.—*Categories and Grading of Histological Abnormalities*

	Non-Specific Changes			
Grade	Fatty change	Inflammatory cell infiltrate	Fibrosis	Cirrhosis
0	< 100 cells per lobule	< 50 cells per portal tract	None	
1	> 100 cells to $\frac{1}{3}$ lobule	> 50 cells per portal tract	Sparse intralobular	
2	$\frac{1}{3}$–$\frac{2}{3}$ lobule	> 100 cells per portal tract	Just bridging portal tracts	Not graded
3	> $\frac{2}{3}$ lobule	> 250 cells per portal tract	Bridging portal tracts	

RESULTS

Untreated Group.—Eleven of the 25 untreated patients had completely normal liver function tests and biopsies. Eight patients had one or more abnormal function tests. Serum amino-transferase was raised in one, alkaline phosphatase in 2, and BSP retention was abnormal in 8 (30% in one; 19% in 2; and between 11 and 15% in the remainder).

Fourteen patients had normal biopsies, and 11 had abnormal biopsies. Of these 11, 7 had non-specific changes and 4 had fibrosis, one of whom was a heavy drinker. None had cirrhosis (see Table III). No specific cause for fibrosis was evident in the others, but all 3 had severe psoriasis of 20 years or more duration.

Treated Group.—Sixteen of the 42 treated patients had completely normal liver function tests and biopsies. Twenty-one patients had one or more abnormal liver function tests. Serum amino-transferase was raised in 10, alkaline phosphatase in 7, and BSP retention was abnormal in 18 (over 20% in 5; over 10% in 8).

Seventeen patients had normal biopsies. Of the 25 with abnormal biopsies, 10 had non-specific changes, 12 showed fibrosis, and cirrhosis was present in 3. The cirrhosis was micronodular in 2 (one of whom had evidence of active damage),

TABLE III.—*Findings in the 25 Untreated Patients*

No. of patients	Histology	Number with abnormal liver function tests	Heavy alcohol intake
14	Normal	3	1
7	Non-specific changes only*	3	0
4	Fibrosis†	2	1
0	Cirrhosis	—	—

* Three grade 1 fatty change, 3 grade 1 cell infiltrate, 1 grade 1 fat and grade 1 cell infiltrate.
† Three grade 1, one grade 2.

and macronodular in one. All 3 cirrhotics were heavy drinkers. Liver function tests and alcohol intake are related to histological findings in Table IV.

Clinical Findings.—At the time of biopsy 3 of the 25 untreated patients and 6 of the 42 treated patients had hepatomegaly. Amongst the treated group

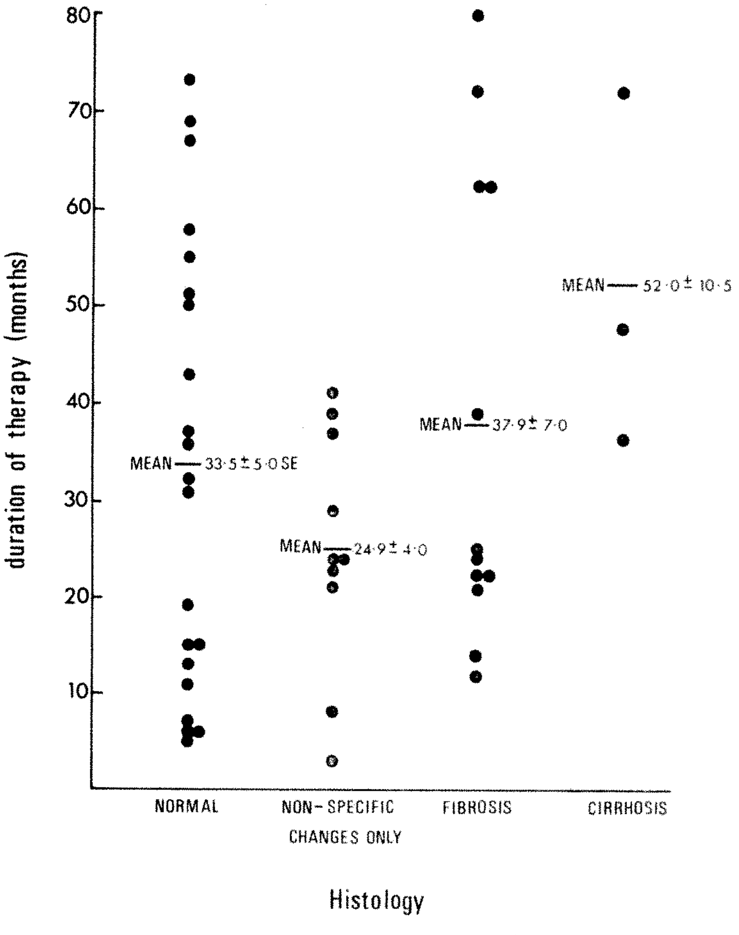

FIG. 2.—Duration of therapy related to histological findings.

TABLE IV.—*Findings in 42 Treated Patients*

No. of Patients	Histology	Number with abnormal liver function tests	Heavy alcohol intake
17	Normal	4	2
10	Non-specific changes only*	7	2
12	Fibrosis†	7	0
3	Cirrhosis	3	3

* Four grade 1 fatty change, 1 grade 2 fatty change, 4 grade 1 cell infiltrate, 1 grade 1 fat and grade 1 cell infiltrate.

† Eleven grade 1, 1 grade 2.

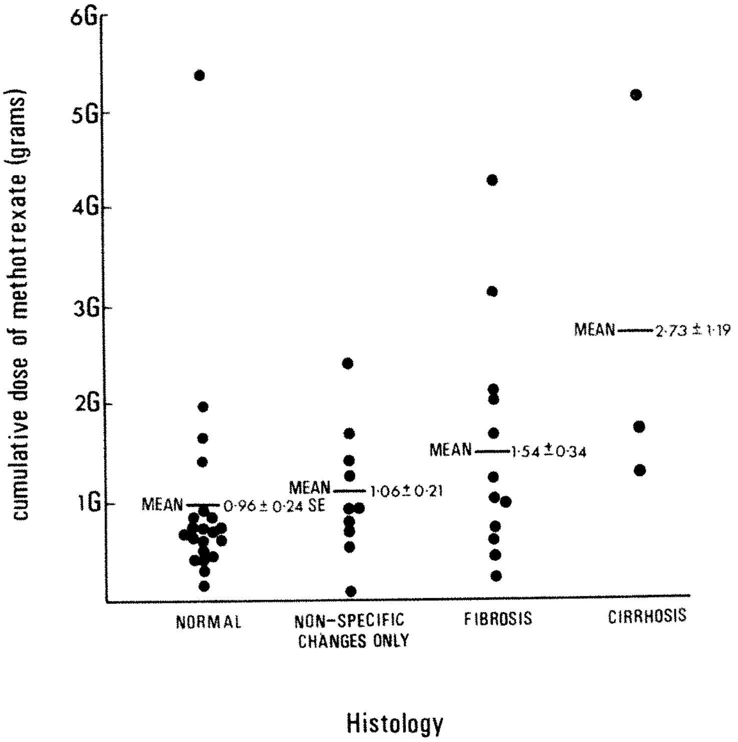

FIG. 3.—Cumulative dosage related to histological findings.

all 3 patients with cirrhosis had hepatomegaly; one of these had splenomegaly and ascites. One other treated patient with portal fibrosis had only splenomegaly. Clinical evidence of portal hypertension was not found in the remainder with liver disease.

Cumulative Dose.—The mean cumulative dose of methotrexate was significantly greater ($P = 0{\cdot}05$) in the patients with fibrosis and cirrhosis than in those with normal biopsies (Fig. 2). However, the patient who had received most methotrexate (5·35 g by the daily oral regime) had a normal biopsy, although the majority of those with normal biopsies had received less than 1·0 g of the drug.

Duration of Therapy.—There were no statistically significant differences in the duration of methotrexate therapy between patients with normal biopsies and those with abnormalities (Fig. 3). However, the 3 patients with cirrhosis had received methotrexate for a mean period of 52 months, in comparison with a mean of 33 months in those with normal biopsies.

Six of the 25 patients with abnormal biopsies had not received methotrexate at the time of biopsy for periods ranging from 1 to 20 months; this group comprised one with fibrosis (no drug for 20 months) and one with cirrhosis (no drug for 7 months).

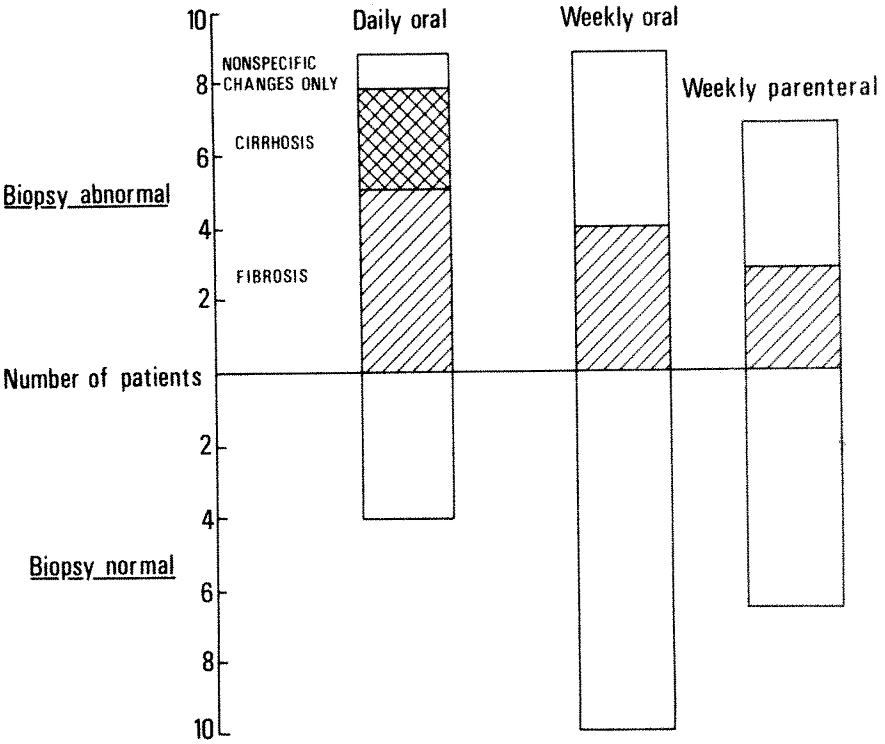

FIG. 4.—Modes of administration related to biopsy abnormalities.

Mode of Administration.—All 3 patients with cirrhosis had received methotrexate predominantly by the " daily oral " regime. Fibrosis was found with approximately equal frequency with all 3 regimes. When fibrosis and cirrhosis were considered together, patients treated by the daily oral regime showed the highest incidence of these abnormalities (although the duration of therapy and cumulative doses of drug were not significantly different in the 3 treatment regimes (Fig. 4)). However, a modified analysis of variance failed to identify a significant excess in the incidence of fibrosis or cirrhosis, considered separately, with the daily oral regime.

Follow-up Biopsies.—Thirty treated patients were re-biopsied (5 re-biopsied twice) at mean intervals of 13 months. Of 15 with previously normal biopsies, 3 developed abnormalities (all grade 1 fibrosis). Of 15 with previously abnormal biopsies, 6 showed improvement; in 2, non-specific changes returned to normal;

and in 4, grade 1 fibrosis was replaced by non-specific change only (2 of the latter had remained on methotrexate).

Prospective Study.—Fourteen of the 25 untreated patients were subsequently given methotrexate. Of these, 11 had normal pre-treatment biopsies and 2 had grade 1 non-specific changes. One patient had fibrosis. Nine have been re-biopsied (6 twice). Two have developed mild abnormalities. The patient with fibrosis (discovered in 1965) was treated with methotrexate before fears of hepatotoxicity arose. After 48 months of therapy a second biopsy revealed cirrhosis. Methotrexate was stopped, but 23 months later a third biopsy still showed cirrhosis. This patient was an alcoholic. The histological changes, cumulative doses of methotrexate and duration of therapy in the 9 re-biopsied patients are summarized in Table V.

TABLE V.—*Prospective Study* (*9 Patients Rebiopsied*)

	Post-treatment 1st Biopsy			Post-treatment 2nd Biopsy		
Pre-treatment histology	Histology	Interval*	Cumulative dose†	Histology	Interval‡	Cumulative dose†
1. Normal	Normal	7	365	Normal	20	1030
2. Normal	Cell infiltrate Grade 1	8	537	Normal	25	1497
3. Normal	Normal	9	390	Normal	15	640
4. Normal	Normal	11	625	—	—	—
5. Normal	Fatty change grade 2, plus cell infiltrate grade 1, and fibrosis grade 1	12	425	Fatty change grade 1, plus cell infiltrate grade 1, and fibrosis grade 1	24	842
6. Normal	Normal	12	450	—	—	—
7. Normal	Normal	12	445	—	—	—
8. Normal	Normal	13	192	Normal	21	192
9. Fibrosis grade 1	Cirrhosis	48	1740	Cirrhosis	71	1740

* Months between commencing treatment and 1st biopsy or ‡2nd biopsy.
† Mg methotrexate since commencing treatment.

DISCUSSION

This study shows that psoriatic patients never treated with methotrexate may show abnormalities of hepatic structure, and the possible hepatotoxicity of methotrexate must be assessed against this background. Unless the incidence of fibrosis and cirrhosis is substantially greater in treated patients, a prospective study is required to assess the hazard.

Our retrospective study shows a trend towards the development of histological abnormality with increasing cumulative dosage of methotrexate. Our prospective study to date shows little histological abnormality, except in 1 patient, who developed cirrhosis, but who was an alcoholic and had fibrosis on a pre-treatment biopsy.

Others have already recorded structural abnormalities of the liver in psoriatic patients never subjected to folic acid antagonists. Over the past 2 years histological findings in well over 100 patients not treated with methotrexate have been reported (Berge *et al.*, 1970; Weinstein *et al.*, 1970; Dahl *et al.*, 1971; Roenigk *et al.*, 1971; Palmer and Sneddon, 1971; Zachariae and Schiödt, 1971; Nyfors *et al.*, 1971), re-affirming the earlier reports of Dogliotti *et al.* (1954) and Huriez *et al.* (1957). Combining these recent reports of untreated patients with our own findings, only 36% of livers were considered histologically normal. The calculated incidence of non-specific change alone is 57%, portal fibrosis 5%, and cirrhosis 2%. Whether these histological findings represent a psoriatic hepatopathy or have other causes cannot be stated. The incidence of these changes in the population is not known. The incidence of cirrhosis (of all types) in recent years, in a survey of 15,000 unselected patients on admission to a large hospital in this country, was estimated to be 0·17% (Stone *et al.*, 1968).

Over the past 2 years histological findings have been reported in 196 methotrexate-treated psoriatic patients (Weinstein *et al.*, 1970; Dahl *et al.*, 1971; Nyfors *et al.*, 1971; Palmer and Sneddon, 1971; Roenigk *et al.*, 1971; Zachariae and Schiödt, 1971). Combining their data with our own, the incidence of non-specific change only is 50%, fibrosis 15%, and cirrhosis 11%. Twelve of 22 treated patients reported to have cirrhosis were known to be moderate or heavy drinkers. We encountered cirrhosis in only 3 patients, all of whom were heavy drinkers, and alcohol may be an important additive factor in the production of liver damage. Synergism of carbon tetrachloride and azathioprine in inducing hepatotoxicity in rats has been reported (Reuber, 1970). Only Dahl *et al.* (1971) have reported many cases of cirrhosis in patients said not be heavy drinkers.

Whilst hepatic fibrosis is usually irreversible, our data suggest that methotrexate-induced fibrosis may, in part at least, resolve on discontinuation of the drug, as may experimentally-induced fibrosis in animals (Hutter *et al.*, 1960; Popper and Udenfriend, 1970).

Clinical and standard biochemical assessment had limited value in reflecting hepatic damage; it is unlikely that isoenzyme studies of alkaline phosphatase would have helped substantially. For this reason we feel that a liver biopsy should precede therapy and should be repeated annually, provided a competent operator is available.

There is no effective substitute for methotrexate in the treatment of the most severe form of psoriasis. However, every effort must be made to limit the dose and duration of therapy so that the risk of irreversible hepatic damage may be minimized.

We thank the physicians to St. John's Hospital for Diseases of the Skin for access to their patients and notes. Mrs Hannaford, medical artist, kindly prepared the figures. These and the tables were photographed by the Department of Photography, London Hospital. We wish to thank those dermatologists who have referred patients to one or other of us. One of us (H.B.) was in receipt of a Medical Research Council grant.

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DOUBLE-BLIND TRIAL OF ZINC SULPHATE IN THE TREATMENT OF CHRONIC VENOUS LEG ULCERATION

M. W. GREAVES AND F. A. IVE

Summary.—The results of a double-blind trial of zinc sulphate in patients with chronic venous leg ulceration are reported. No significantly increased rate of healing was observed in the zinc-treated group.

LOWERED plasma zinc concentrations in patients with venous leg ulceration have now been reported in several studies (Greaves and Boyde, 1967; Withers *et al.*, 1968; Greaves and Skillen, 1970). In this paper we describe the results of a double-blind trial of oral zinc sulphate treatment in patients with this condition.

METHODS

Patients.—A total of 38 patients (28 female) with chronic venous leg ulceration who were attending the Department of Dermatology of either the Royal Victoria infirmary, Newcastle upon Tyne or the Dryburn Hospital, Durham, were studied. The nature of the trial was explained to all patients and their informed consent obtained.

Patients were admitted to the trial if their ulceration was considered to be predominantly due to venous stasis on the grounds of presence of varicose veins, oedema and hyperpigmentation. In all cases the ulcer had failed to respond to previous topical treatment. Associated disease was present in 5 patients (diabetes 3; psoriasis 1; rheumatoid arthritis 1). All but 5 of the patients were ambulant. Patients admitted to the trial were interviewed by one of us at the first and all subsequent attendances.

Treatment.—Identical capsules containing either 220 mg $ZnSO_4.7H_2O$ or lactose were obtained from Medo Chemicals Ltd., London, N.W.5. Patients were randomly allocated to either placebo or zinc capsules by the Pharmacy Department, Royal Victoria Infirmary, Newcastle upon Tyne. They were instructed to swallow one capsule 3 times daily, after breakfast, after the mid-day meal and after the evening meal. Thus, neither the physician nor the patient knew which capsules had been prescribed. The capsules were taken until the ulcer had healed or until 4 months had elapsed, whichever occurred first. Throughout the period of the trial the ulcers were treated topically with simple antiseptic lotions, non-adhesive dressings and a crepe bandage. No systemic treatment other than the zinc or placebo capsules was prescribed. Patients attended every 3–4 weeks during the trial, and at each attendance verbal confirmation that the capsules were being taken regularly was obtained.

Measurement of effect of treatment.—The outline of the area of ulceration was traced on transparent paper at first attendance, and this procedure was repeated at the end of the trial on any remaining area of ulceration. An approximate estimate of the rate of healing was made by one of us (M.W.G.), using the following method. On the initial tracing 2 radii at right angles were measured, one of which lay along the long axis of the ulcer. If there was any remaining ulceration, similar measurements were made on the post-treatment tracing. The mean difference between the 2 pairs of corresponding radii represents the extent of linear re-epithelialization during the treatment period.

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The mean rate of healing per week was determined for each patient by dividing total linear re-epithelialization by time taken to total healing; or, if healing was incomplete, to the end of the trial. When more than one ulcer was present, the results were averaged.

RESULTS

Thirty-six patients completed the trial, 2 having defaulted.

No patients complained of any side effects. The results are summarized in Table I. Although the mean rate of linear re-epithelialization was greater in the zinc-treated group, the difference did not reach statistical significance.

TABLE I.—*Rate of Linear Re-epithelialization in Control and Zinc Groups*

	Zinc	Placebo
No. of patients	18	18
Mean age (years)	63 ± 3 SEM	64 ± 3 SEM
Mean area of ulceration cm^2)	34·6 ± 17·0 SEM	32·3 ± 13·2 SEM
No. showing complete healing	3	2
Mean linear healing rate (mm/week)	0·9 ± 0·3 SEM	0·6 ± 0·2

The difference between the mean linear healing rates for the 2 groups is not significant ($t = 1 \cdot 3$; $0 \cdot 3 > p > 0 \cdot 2$).

DISCUSSION

Although no satisfactory controlled trials have been published, a number of workers have suggested that oral zinc sulphate treatment accelerates healing of chronic cutaneous ulceration of various types, including leg ulcers (Husain, 1969; Serjeant *et al.*, 1970) and bed sores (Cohen, 1968), although others have been unable to demonstrate this effect (Myers and Cherry, 1970). Greaves and Skillen (1970) recently reported the effects of long-continued ingestion of zinc sulphate in patients with venous leg ulceration. Although their study was designed primarily to investigate plasma zinc concentrations before and after treatment, and side-effects of zinc treatment, these authors noted that some degree of healing occurred in all patients studied. The present double-blind study does not support these preliminary observations, since no significantly increased rate of healing occurred in the zinc-treated group compared with the control groups. The dosage of zinc given is known to raise the plasma zinc concentrations by about 25% in the majority of patients (Greaves and Skillen, 1970). It is therefore concluded that there is no justification for administration of zinc in this form in patients with chronic venous leg ulceration. A recent study (Neathery *et al.*, 1972) showed that the ligand with which zinc is combined materially affects tissue distribution of orally administered zinc. Zinc combined with an organic ligand gave significantly higher tissue levels of zinc than zinc chloride although absorption of the two forms was equal. Better results might have been obtained in the present study if a zinc-protein complex had been given.

Postscript

After this article had gone to press, T. Hallbrook and E. Lanner have reported a double-blind trial of zinc sulphate in patients with venous leg ulcers (*Lancet*, October 14th, 1972). They have confirmed our earlier findings of low plasma zincs in patients with venous leg ulcerations. They also found that oral zinc sulphate accelerated the rate of healing of patients with venous leg ulcers who have a low plasma zinc concentration. We did not determine the plasma zinc concentrations in our patients which may explain the negative results of our study.

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Case Reports

Br. J. Derm. (1972) **87**, 635.

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CONGENITAL MACULAR URTICARIA PIGMENTOSA COMPLICATED BY MASSIVE NODULAR MASTOCYTOSIS WITH SYSTEMIC INVOLVEMENT

LUCIO BAKOS, RONILDO STORCK AND ERNESTO SILVEIRA NETTO

Summary.—A case of congenital macular urticaria pigmentosa ran a normal course until puberty, when large cutaneous tumours appeared, associated with episodic flushing, malaise and gastrointestinal symptoms. There were repeated episodes of profuse bleeding after local trauma. Systemic mastocyte infiltrate was found in liver, lymph nodes and bones. Review of the literature on mastocytosis failed to show any similar case.

Although the patient is now aged 37 and has survived surgical removal of cutaneous tumour masses, it is suggested that her prognosis is poor.

THE natural course of infantile urticaria pigmentosa is benign and closely related to the age of onset of the disease. Most cases develop the eruption in infancy or early childhood and the lesions disappear entirely by adolescence or early adult life. In a few cases the lesions persist in a milder form throughout life.

The nodular form of the disease tends to disappear more rapidly than the maculopapular form, and is not usually associated with systemic involvement.

This report describes a case of congenital urticaria pigmentosa, the course of which did not follow the usual benign pattern.

CASE REPORT

At birth a white girl, with no family history of skin disorder or allergy, presented the pink and pigmented macules of urticaria pigmentosa all over the skin surface except the face, palms and soles. The lesions sometimes became swollen and raised, mainly after scratching. Pruritus was mild.

At puberty large tumours developed on several parts of the skin (Fig. 1), the largest being on the thighs. Associated with the development of these tumours the patient started to complain of episodes of weakness, flushing, malaise, dyspnoea, nausea, vomiting and diarrhoea. She showed easy bruising, weight loss and slight enlargement of the abdomen. Episodes of profuse bleeding from the tumours followed minor local trauma. Some of these tumours, especially on the abdomen, spontaneously remitted, leaving sharply demarcated areas of atrophy of the skin.

At the age of 37, the patient showed evidence of weight loss, slight ascites, hepatosplenomegaly (liver palpable 10 cm below the costal margin), generalized lymphadenopathy and muscle wasting. Persistent facial flushing was present. On the skin

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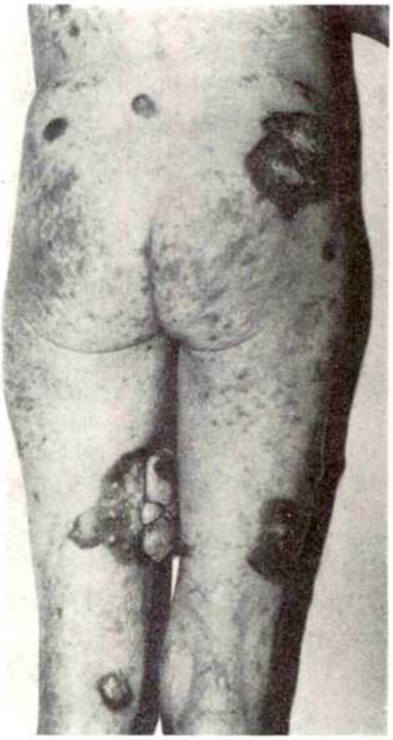

FIG. 1.—Back view of the patient, showing macules and tumours.

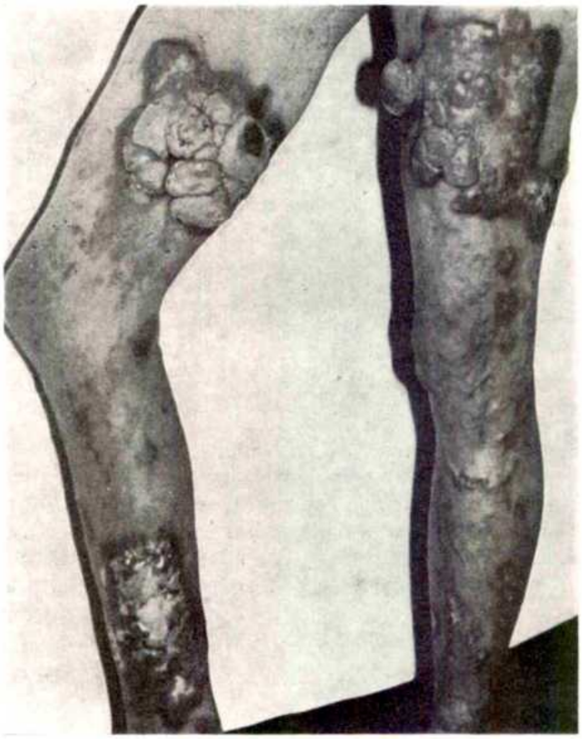

FIG. 2.—Tumour masses on thighs.

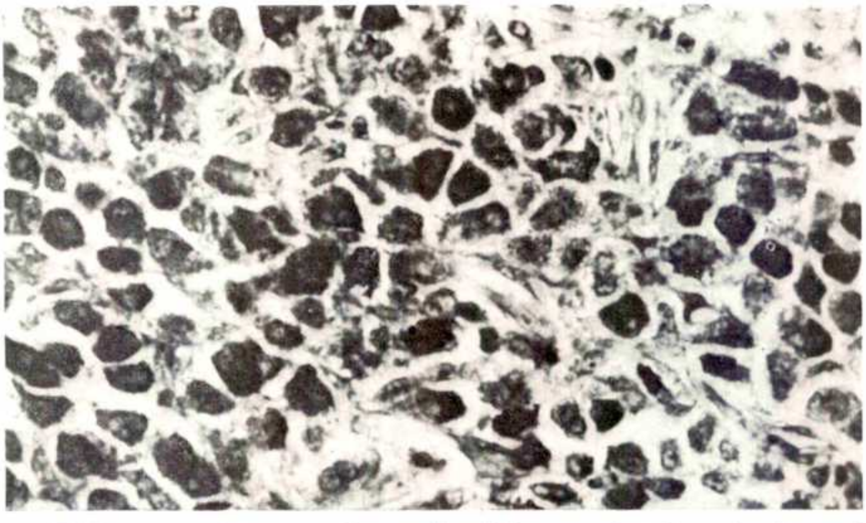

FIG. 3.—Thigh tumour. Aggregates of mast cells, with large number of cytoplasmic granules (Giemsa × 600).

there was a widespread eruption of reddish or brown macules of urticaria pigmentosa, some with a purpuric component, and often surrounded by a network of telangiectases. These urticated on rubbing. The face, palms and soles were spared. Localized areas of skin atrophy were present on the abdomen at the site of lesions which had spontaneously resolved. Hair, nails and mucous membranes were normal.

The most remarkable finding was the presence of large tumours on both thighs, the legs, abdomen, lumbar region, right buttock, right forearm and on the posterior aspects of both arms. The largest were located on the thighs (Fig. 2) and measured 15 and 16 cm. These lesions showed sharply demarcated borders, and their surface was grossly irregular and lobulated; it was smooth, but showed some erosions and crusting. These tumour masses were mainly flesh-coloured, with some areas of dark brown pigmentation. There were dilated blood vessels scattered on the surface, and areas of scarring. The tumour masses were firm, attached to skin and fixed to the underlying structures. The other smaller tumours had the same consistency and nodular form, but were more pigmented and had no erosions or scarring.

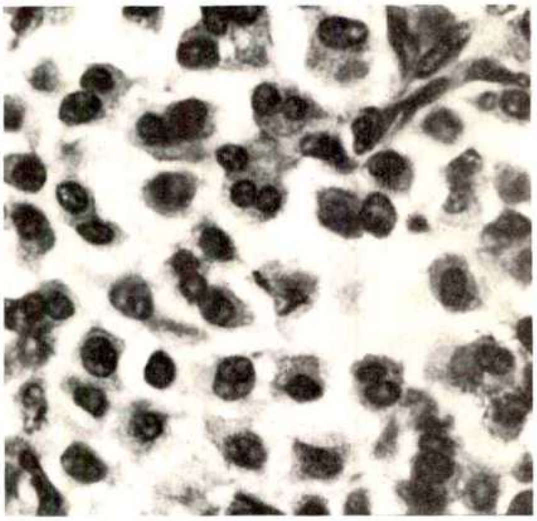

FIG. 4.—Thigh tumour. Mast cells, with multilobulated nuclei (H. and E. × 600).

Investigations

Hb: 9·4 g %; WBC: 38,000/mm^{-3}, 2,300 eosinophils/mm^{-3}, but no mast cells. Platelet count, bleeding, clotting and prothrombin time were all normal. Urine showed slight proteinuria; microscopy was normal.

Serum proteins, total and electrophoresis, SGOT, SGPT and alkaline phosphatase were all normal. Acid phosphatase was 7 Bodanski units. ECG was within normal limits.

X-ray examination of vertebrae showed diffuse osteoporosis. There was a nodular condensation on the tenth right rib, surrounded by areas of focal reabsorption. There were small osteolytic lesions of the skull. Chest x-ray showed bilateral interstitial fibrosis of lungs.

Laparoscopy.—Laparoscopy showed marked enlargement of the liver, more pronounced in the right lobe, with several fibrous bands crossing its surface, giving the organ a grossly lobulated appearance. The colour was pale red, with several bright red spots scattered over all lobes. The spleen was not visualized. The gall-bladder was normal.

Histology.—The macular lesions showed epidermal melanin pigmentation, pigmentary incontinence and many grossly dilated blood vessels in the dermis, which were surrounded by mainly fusiform mast cells, with some eosinophils and lymphocytes.

The tumours were formed of dense aggregates of closely packed polyhedral or cuboid mast cells, with round or oval nuclei and pale eosinophilic cytoplasm. Giemsa and methylene blue stains showed large amounts of metachromatic cytoplasmic granules, sometimes obscuring the nuclei (Fig. 3). Some of these mast cells had large and multilobulated nuclei (Fig. 4), without any mitotic figures or other abnormalities. The infiltrate was dense, intermingled with many eosinophils and lymphocytes, and in the mass of the tumour there was a profusion of large ectatic blood vessels with thick walls (Fig. 5). The infiltrate extended from direct contact with the epidermis through the deeper parts of dermis and subcutis and infiltrated underlying skeletal muscle (Fig. 6). The epidermis showed slight acanthosis and there were increased amounts of melanin in the basal layer and in melanophores in the upper dermis.

Liver biopsy showed proliferation of connective tissue in portal spaces, often surrounding bile ducts and containing a round cell infiltrate, with mast cells, lymphocytes and some histiocytes. Some sinusoids showed scattered mast cells on their edges.

Lymph nodes showed excessive numbers of mast cells in the subcapsular and medullary sinuses and in some germinal centres.

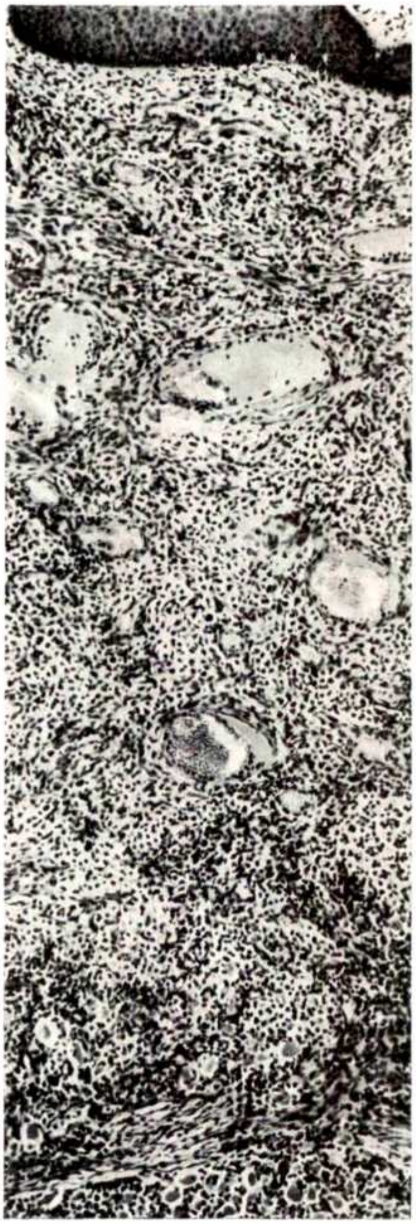

FIG. 5.—Ectatic blood vessels in the mass of the tumour. (H. and E. × 160).

Operation

At the age of 37 both thigh lesions were excised with skin grafting, to stop the episodes of bleeding. During surgery she had a marked fall of blood pressure due to manipulation of tumours. Following operation the episodes of malaise, flushing and gastrointestinal symptoms became less frequent.

DISCUSSION

Urticaria pigmentosa or cutaneous mastocytosis can have its onset in infancy, childhood or adult life. In 75% of cases the onset is before puberty, most of them showing the first lesions before the age of 6 months (Caplan, 1963).

In children the disorder may appear either as a solitary lesion or as a disseminate eruption of macules, papules or nodules. The eruption is usually monomorphous, but it may be mixed. Blistering is not unusual. Other less frequent varieties such as the telangiectatic and diffuse cutaneous forms occur (Sagher and Even-Paz, 1967).

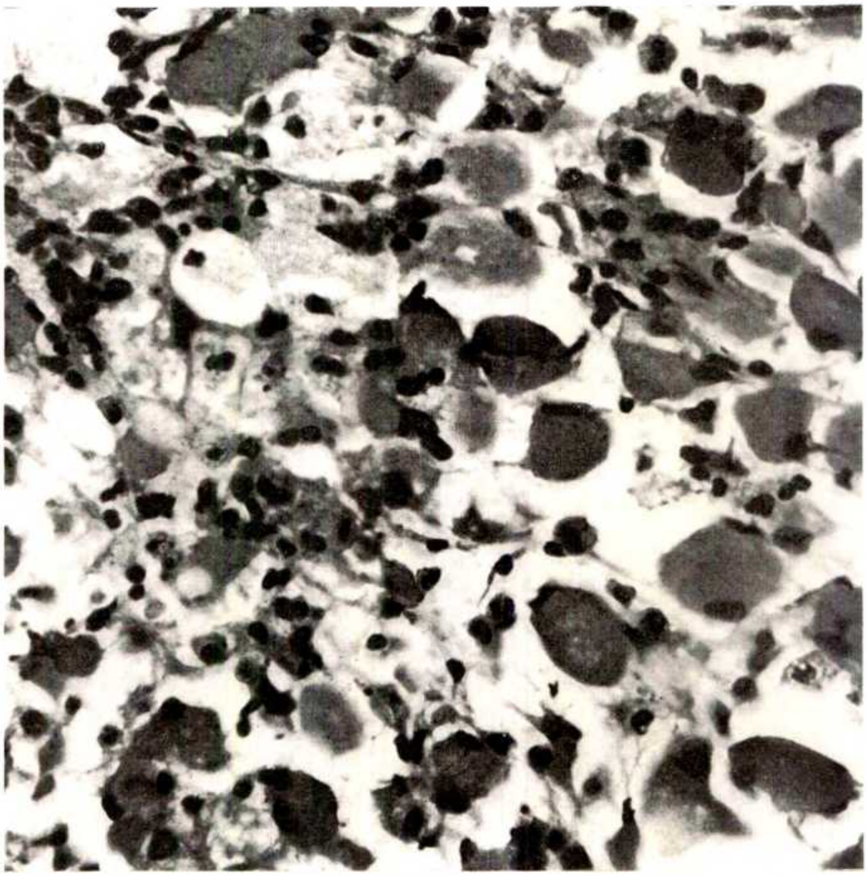

FIG. 6.—Infiltration of skeletal muscle by mast cells. (H. and E. × 600).

The natural history of urticaria pigmentosa in children has been well reviewed (Johnson and Helwig, 1961; Klaus and Winkelmann, 1962; Caplan, 1963; Niordson, 1962; Sagher and Even-Paz, 1967).

Solitary mastocytomata or mast cell naevi have the most favourable prognosis, for they tend to disappear spontaneously after a few years (Johnson and Helwig, 1961; Klaus and Winkelmann, 1962; Caplan, 1963).

The disseminate form with onset in infancy or early childhood also deserves a good prognosis, since about half of these patients will clear completely by adolescence or early adult life, sometimes within a few years after the onset of the disease. Of the remainder, 30% show some resolution of the cutaneous eruption; and in

20% it persists unchanged, but causes few symptoms (Caplan, 1963; Niordson, 1962).

When the onset is in later childhood, but before puberty, although the lesions show less tendency to disappear completely (Niordson, 1962; Caplan, 1963), the risk of systemic involvement seems not to be greater. About 60% of cases of disseminated cutaneous mastocytosis have their onset in early life. However, the risk of systemic involvement is 8 times greater in those whose disease begins after puberty (Sagher and Even-Paz, 1967).

The nodular form clears more rapidly than the maculopapular one, and it is not usually associated with systemic involvement (Klaus and Winkelmann, 1962) although this may occur in rare cases (Ellis, 1949). The benign course is illustrated by the patient presented by Kierland and Stegmaier (1950). This patient had nodules at birth, and infiltration had disappeared in one-third of them after a period of approximately 4 years. Caplan (1963), describing mastocytosis of onset in infancy or early childhood, includes 5 cases of nodular or mixed nodular forms in his group of 72 patients. All of them showed spontaneous resolution. In Sagher and Even-Paz's review (1967) of 145 cases of systemic mastocytosis, only 3 developed from the nodular infantile form.

Thus, although there are some cases which have a poor prognosis, as a general rule urticaria pigmentosa may be regarded as a benign and usually self-limiting form of mastocytosis with a tendency to clear with age, this course being influenced by the age of onset of the disorder.

Regarding the transformation of mastocytosis macules into more solid lesions, it is known that, for a variable period of time, the lesions can increase in number and can become more raised and bigger (Caplan, 1963; Sagher and Even-Paz, 1967), but we have found no record of the development of larger tumour masses, although plaque-like lesions (Gross and Hashimoto, 1964; Carr, 1971) and verrucous changes (Goldsmith and Wells, 1952) have been reported.

The course of the disease in our patient thus contrasts strikingly with the usual evolution of infantile urticaria pigmentosa. She had the disseminate macular form present from birth; at puberty, however, the lesions did not resolve, but started to increase in number, and she developed enormous mast cell tumours on the sites of formerly macular lesions, associated with symptoms suggesting episodic release of histamine. Biopsies of liver and lymph node, as well as bone x-rays, showed systemic involvement. This sequence of events is apparently unique.

Histological features also were unusual in this patient. Telangiectatic capillaries, venules and lymphatics may be seen in mastocytosis, especially in the form known as telangiectasia macularis eruptiva perstans (Sagher and Even-Paz, 1967), probably due to a long-standing continuous dilatation and congestion of dermal vessels by histamine release (Demis *et al.*, 1961), but large ectatic blood vessels as in this patient have not been described previously (Winkelmann, 1971).

Multinucleated mast cells are rare, but have been described in nodular mastocytosis (Kierland and Stegmaier, 1950; Prado, 1969). The infiltrate may extend into the subcutis (Sagher and Even-Paz, 1967), but infiltration of muscle, although seen in dogs (Head, 1958), has not been described in man.

Systemic involvement in our case was found in the most frequently affected sites (bones, liver, lymph nodes). Sternal marrow, although affected in 90% of these cases, did not show increased numbers of mast cells in 2 needle aspirations.

This procedure, however, is not enough to rule out marrow involvement. Bone biopsy gives more frequent positive findings (Johnstone, 1954).

Anaemia is the most common abnormality of the peripheral blood, being present in some 40% of patients with the systemic form. Leukocytosis has been found in 18%, and in 12% eosinophilia up to 65%. In one-third of patients presenting eosinophilia the outcome has been fatal (Sagher and Even-Paz, 1967).

About one-third of the patients with systemic involvement develop a related malignant disease, most commonly a leukaemia or reticulosis. Mast cell leukaemia has been reported by Efrati (1957). Sagher and Even-Paz (1967) state that 48% of patients with malignant mastocytosis die within 2 years of the onset of the disease, although 12% can have a survival ranging from 21 to 30 years.

Although our patient has survived for 37 years, the progressive course of her disease, the blood eosinophilia and the presence of systemic involvement, suggest that the prognosis is now poor.

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AN UNUSUAL PRESENTATION OF DISCOID LUPUS ERYTHEMATOSUS

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Summary.—An unusual presentation of discoid lupus erythematosus in a woman aged 32 is described. Typical plaques of discoid lupus erythematosus were associated with an atypical, widespread, discrete, umbilicated papular eruption on the back, resulting in hypertrophic follicular scars of a type sometimes seen in acne vulgaris. Persistent pruritus was also a feature.

Discoid lupus erythematosus usually affects the exposed parts, e.g. face, scalp, ears and nose. Covered areas such as the trunk, arms and legs are relatively less often involved. Commonly the eruption presents as erythematous patches of various sizes, with adherent scale, follicular plugs, telangiectases and depressed scars. It may, however, be manifested in a warty, annular, profundus, telangiectatic, bullous, ulcerated, hyperkeratotic or erythema-multiforme-like form (Rowell, 1968). Pruritus is not a prominent feature of discoid lupus erythematosus.

The case described below is very unusual in that the patient has typical plaques of discoid lupus erythematosus of face and scalp, associated with an atypical, widespread, chronic, discrete, papular eruption on the back, resulting in hypertrophic follicular scars of a type sometimes seen in acne vulgaris. Persistent pruritus is also present, waxing and waning in intensity over the years.

As far as we are aware there has been no such case described—at least, not in the English language literature.

CASE REPORT

The patient, a woman aged 28, was first seen at the Glasgow Royal Infirmary in April 1969, with pruritus and a typical eruption of the face for the past four years, and with lesions of the scalp, giving rise to cicatricial alopecia. In addition there was a widespread eruption of discrete papules on the back, shoulders and upper arms (Fig. 1, 2). The nature of this eruption was not apparent at first on clinical examination. She was admitted to hospital for further investigation. Apart from an E.S.R. of 58–75 mm/1st hour, other investigations proved negative. There was no clinical or laboratory evidence of systemic lupus erythematosus. No member of the family was known to suffer from systemic or discoid lupus erythematosus. There was no relevant history of drugs which could be incriminated in the production of the eruption.

She was treated with topical steroids, sun barrier creams, and oral antihistamines, with some benefit. She became pregnant in July, 1969. This did not influence her eruption in any way, but she had a miscarriage at about 24 weeks. There was a history of a previous miscarriage, followed by the birth of 2 live girls.

As time passed, the eruption on the face, scalp and back gradually spread. The evolution of lesions on the back was interesting. These started as widespread, discrete, erythematous follicular papules, with a prominent central scale. On healing, they left

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well demarcated, skin-coloured, dome-shaped scars, with a central crater (Fig. 2). Some of them were covered with telangiectases.

She became pregnant again in July 1971 and was re-admitted in November 1971, with severe generalized pruritus. Apart from a recent candidal infection of the genital tract, successfully treated with nystatin, there was no discernible cause for the exacerbation of her pruritus. She denied any systemic upset, and general examination was negative.

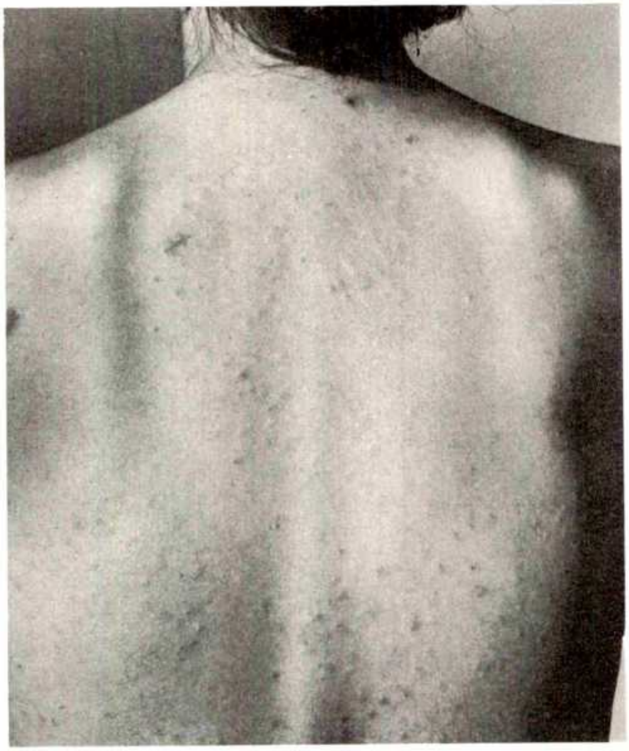

FIG. 1.—Widespread, discrete, papular eruption of discoid lupus erythematosus on back, resulting in hypertrophic follicular scars.

The results of the investigations which have followed a similar pattern throughout, were as follows:

Hb 11·7 g;W.B.C. 6600/mm^3; platelets 170,000/mm^3; E.S.R. 50 mm/1st hour; blood film—rouleaux formation, anisocytosis, normochromia and slight polychromasia. Tests for liver function, serum proteins, blood sugar, urea, electrolytes, L.E. cell phenomenon, L.E. latex, W.R., rheumatoid factor, antinuclear factor, antithyroid and gastric parietal cell antibodies, E.C.G. and urinanalysis for blood, albumin and sugar were all negative. Chest x-ray was not taken so as to avoid unnecessary exposure to x-rays during pregnancy. A previous radiological examination in 1969 had been normal. Test for cold agglutinins and Coomb's test were negative. Mantoux test was strongly positive to a dilution of 1 in 1000.

Histology

1969—Biopsies from the back and scalp showed marked hyperkeratosis and follicular plugs with some mild, patchy atrophy of the epidermis. There was basal cell liquefaction present, mainly around the hair follicles, but also in the epidermis. The upper dermis showed minimal oedema, with a slight, mainly perifollicular, lymphocytic infiltrate. The appearances were consistent with a diagnosis of discoid lupus erythematosus.

1971—Biopsy of the atypically affected skin of back showed similar changes (Fig. 3).

Immunofluorescent Test for L.E.

This was positive in affected skin on the back, but not in normal skin.

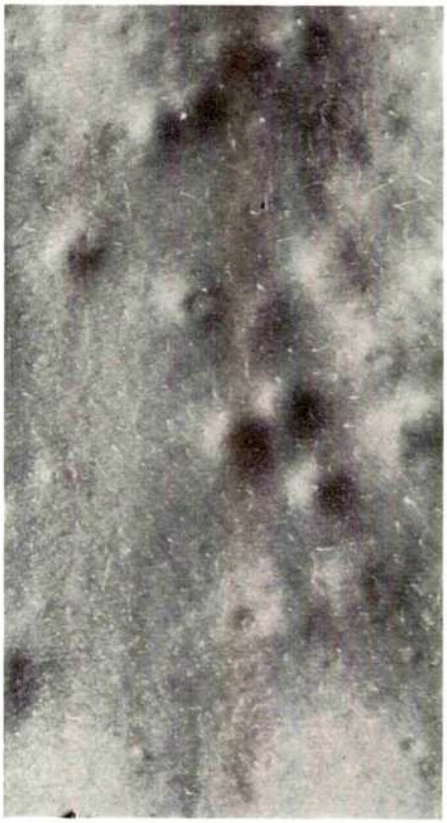

FIG. 2.—Close up of discrete follicular lesions of discoid lupus erythematosus on back.

DISCUSSION

There is no doubt that this patient has typical discoid lupus erythematosus of the face and scalp, but the follicular eruption on the back, resulting in discrete, umbilicated, dome-shaped scars involving almost the entire back, is unusual. Certainly in the absence of facial and scalp lesions it would have been impossible to consider a clinical diagnosis of discoid lupus erythematosus.

It is now well established by direct immunofluorescent techniques that active lesions of both discoid and systemic lupus erythematosus show the presence of immunoglobulins (IgG, IgM and IgA) and complement at the dermo-epidermal junction. The uninvolved skin of patients with systemic lupus erythematosus

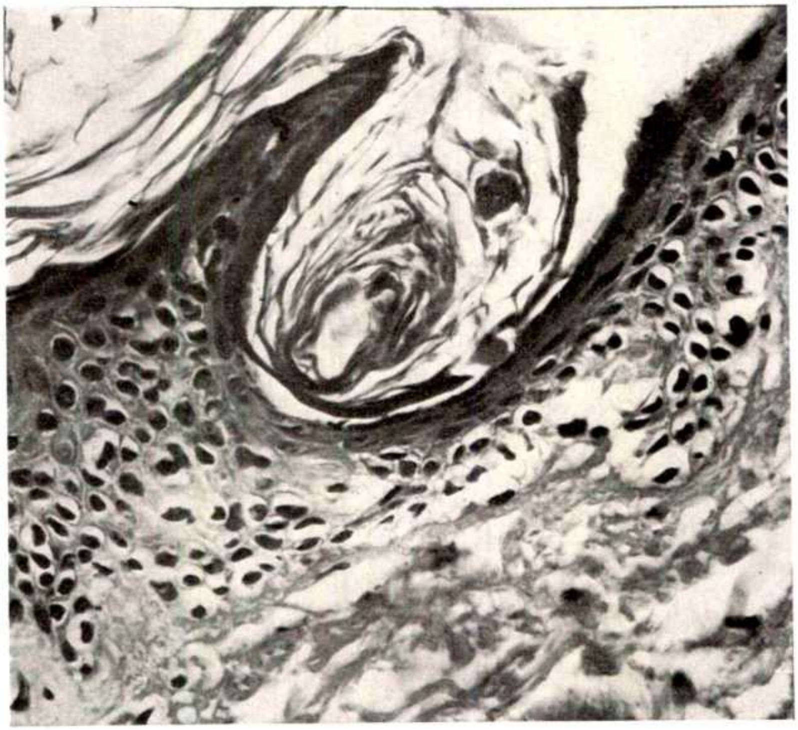

FIG. 3.—Histology of skin from back, showing hyperkeratosis, follicular plugs and basal cell liquefaction (× 580).

yields positive dermo-epidermal fluorescence in a large number of cases, while uninvolved skin of patients with discoid lupus erythematosus is regularly negative (Burnham and Fine, 1969; Kay and Tuffanelli, 1969; Rothfield and March, 1971). Our patient exhibited positive immunofluorescence in affected skin on the back, but none in normal skin, thus confirming the clinical diagnosis of discoid lupus erythematosus.

We wish to thank Dr J. O'D. Alexander, who allowed us to publish this case, and Messrs W. E. Towler and T. Parker for their help with the illustrations.

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Quarterly Review

DRUG REACTIONS XX

Cutaneous Reactions to Anticonvulsants

The standard drugs used in the treatment of epilepsy include the derivatives of hydantoin, oxazolidine, and succinimide. Phenobarbitone and Primidone, which is chemically related to the barbiturates, are both very useful anticonvulsants. The cutaneous reactions to barbiturates have been fully discussed in a previous review (Almeyda and Levantine, 1972). Carbamazepine is mentioned because of its use in trigeminal neuralgia.

Anticonvulsant drugs are taken regularly for a prolonged period, and should not be stopped until the patient has been free from convulsions for at least 2–3 years. In fact, many patients may have to receive therapy for life, so that it is important to recognize the various side effects.

Hydantoin Derivatives

This group includes phenytoin and methoin. Of these two drugs, the former is the most satisfactory, being useful in grand mal and temporal lobe epilepsy. Cutaneous reactions following the ingestion of hydantoin derivatives are commonly erythematous, scarlatiniform, or morbilliform in character. The eruption tends to appear about the ninth or tenth day of treatment, and may be accompanied by fever (Ritchie and Kolb, 1942). The incidence of such reactions is reported to be between 5% and 10% of patients taking phenytoin, and at least 5% of those receiving methoin (Bailey *et al.*, 1965; Lindmayr, 1951).

Gingival hyperplasia is a well recognized side effect of hydantoin therapy. Babcock (1965) recorded a 36% incidence of this complication in patients receiving phenytoin. He also noted that the incidence was greater in children and young adults. The mechanism of this reaction is thought to be in part a consequence of the drug's ability to stimulate fibroblastic proliferation (Houck, 1963).

A less common, but well documented side effect of hydantoin therapy is hypertrichosis. Livingston *et al.* (1955) reported the occurrence of this in 16 epileptic children, 14 of whom were females. Their ages ranged from 3 to 14 years. The increased hair growth occurred between 2 and 3 months after starting therapy, and predominantly affected the extensor surfaces of the extremities. In some children it appeared on the trunk and face, but there was no true virilization. Three of the children were investigated endocrinologically and no abnormality was found. In 12 of the children, hypertrichosis disappeared within a year of withdrawal of the offending drug.

At least 10% of patients receiving hydantoin preparations, particularly methoin, develop pigmentation of the face and neck resembling chloasma. Kuske and Krebs (1964) investigated 13 such patients and found no significant endocrinological abnormality. They described a method using isolated ends of tails of Xenopus laevis larvae for studying pigment cells, and were able to show a direct effect of hydantoin. Skin biopsies in affected individuals have shown the pigment to be melanin (Hunter and Jenkins, 1951).

A rarer manifestation of hydantoin sensitivity is exfoliative dermatitis (Ritchie and Kolb, 1942). Gropper (1956) reported a fatal case associated with hepatitis. In recent years a hydantoin-induced syndrome has been recognized which is characterized by lymphadenopathy, fever, exfoliative dermatitis and eosinophilia. Robinson and Stone (1970) described a reaction occurring in 8 patients after approximately 14 days of phenytoin therapy. The patients all developed a pyrexia and had generalized lymphadenopathy. There was a widespread, confluent, erythematous macular eruption, and marked facial swelling. As the eruption subsided there was scarlatiniform peeling. All the patients showed marked eosinophilia, an elevated blood urea, and reversal of the albumin globulin ratio. On other occasions an infectious mononucleosus-like syndrome has occurred (Siegal and Berkowitz, 1961), and there are several reports of a malignant lymphoma-like syndrome (Saltzstein and Ackerman, 1959; Oates and Tonge, 1971; Sparberg, 1963). The latter syndrome has been mimicked so closely in some patients that alkylating agents have been prescribed (Rosenfeld *et al.*, 1961). The mechanism of these syndromes is thought to be a hypersensitivity reaction. Robinson *et al.* (1965) demonstrated circulating antibodies against phenytoin in a woman who had a condition simulating Hodgkin's disease. Holland and Mauer (1965) extensively investigated a child with a similar condition and demonstrated lymphocyte transformation on exposure of a culture of the patient's lymphocytes to phenytoin. They could not, however, demonstrate any antibodies against the drug.

Other less common complications attributed to hydantoin therapy include urticaria (Jones, 1951), Stevens-Johnson syndrome (Watts, 1962; Rudner, 1970), a lupus erythematosus-like syndrome (Jacobs, 1963; Lee *et al.*, 1966) toxic epidermal necrolysis (Bailey *et al.*, 1965; Farber *et al.*, 1965), a fixed drug eruption (Goldstein *et al.*, 1963) and periarteritis nodosa (Van Wyk and Hoffman, 1948; Haas, 1967).

Oxazolidine Derivatives

Troxidone, and paramethadione are both oxazolidine compounds. They are indicated in petit mal epilepsy but are not as effective as ethosuximide. Cutaneous reactions are infrequent, the most common being exanthematic eruptions which usually occur at the beginning of the therapy (Woodcock, 1968). Holowach and Sanden (1960) reported 2 patients in whom alopecia developed during troxidone therapy. In each case prompt regrowth of hair occurred when the drug was withdrawn. The type of alopecia was not documented. The other oxazolidine derivative, Primidone, is remarkably free of side effects although one patient developed systemic lupus erythematosus, which remitted on stopping the drug. As with the other anticonvulsants a systemic lupus erythematosus like syndrome has been reported (Lee *et al.*, 1966; Verbov, 1968) and also erythema multiforme (Leblanc and Feldman, 1961).

Succinimide Derivatives

This group of drugs includes ethosuximide which is the drug of choice in petit mal epilepsy. Reported cutaneous side effects are few. Weinstein and Allen (1966) in a review of 87 children receiving this drug, reported a diffuse morbilliform eruption in 2 patients, one of whom had leucopenia. One child developed urticaria which cleared despite continuous medication. Ethosuximide-induced systemic lupus erythematosus has been recorded (Livingston *et al.*, 1968), and this drug has also been implicated as a cause of Stevens–Johnson syndrome (Coursin, 1966).

Carbamezepine

This drug is an iminodibenzyl derivative related to imipramine. In addition to having anticonvulsant properties it is used extensively in the treatment of trigeminal neuralgia. Rashes, which occur in 3 to 4% of patients, may be pruritic, erythematous, scaly, morbilliform or urticarial (Taylor, 1963; Carnaille *et al.*, 1966). On one occasion an itching erythematous rash was followed by purpura and fatal aplastic anaemia (Spillane, 1964). Harman (1967) observed a patient with a pigmented purpuric rash indistinguishable from a carbromal eruption. Other reports of light sensitivity (Miller, 1966), Stevens–Johnson syndrome (Coombes, 1965) and a reversible illness with features of both lupus erythematosus and dermatomyositis (Simpson, 1966) have been recorded.

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INDEX

SUBJECTS

Acne vulgaris; effect of a medicated wash on (letter), 292
— —; effect of clindamycin in—a clinical and laboratory investigation, 37
— —; pilo-sebaceous duct obstruction and, 327
Acneiform eruption, localized; following cobalt irradiation, 274
Acrodermatitis enteropathica with lactose intolerance (case title), 174
Adenosine triphosphate content of guinea-pig skin stored at −196°C, 23
Alopecia, psoriatic (letter), 73
—, traction; due to " hair extension " (letter), 79
American Zoologist, 515
Amoebiasis of the vulva: review of the recent literature on, 64
Ampicillin rashes, 293
Amyloid; the nature, pathogenesis and staining properties of, 280
Angioma (strawberry mark); earliest features of, in the newborn, 138
Antibodies, nickel, 320
Anticonvulsants; cutaneous reactions to, 646
Appointments, 186, 298, 404, 522
Arbeitsmedizin, Sozialmedizin, Arbeitshygiene, 515
Atrophy of the skin, corticosteroid-induced; with telangiectasia, 548
Autoimmunity: pemphigus as an autoimmune disease (letter), 78
Autoradiography: autoradiographic studies in the inflammatory reaction in pityriasis lichenoides, 534
  comparative study of ^{3}H-thymidine labelling of the dermal infiltrate of skin allergic and irritant patch test reactions in man, 460
  human epidermal cell cycle *in vitro*, 13
  pityriasis lichenoides; epidermal component of, 110
Azathioprine in the treatment of pityriasis rubra pilaris, 42

Bacterial infections:
  aetiology of impetigo contagiosa, 420
  chancroid of the vulva: review of recent literature on, 63
  chlamydiae infections of the vulva: review of recent literature on, 59
  erythrasma lesions; quantitative study of, 130
Baldness, female; hair diameter in, 361
Biochemistry:
  acid hydrolases in the human epidermis, 450
  adenosine triphosphate content of guinea-pig skin stored at −196°C, 23
  age and sex variation in skin surface lipid composition and sebum excretion rate, 333
  changes in the fatty acid composition of the phospholipids, triglycerides and free fatty acids with depth in the cow snout epidermis, 227
  cyclic 3′,5′ adenosine monophosphate-dependent protein kinase of skin.
   I. Measurement and properties, 571
   II. Levels in atopic dermatitis and psoriasis, 577
  effect of chronic sun damage on the activity of metabolic enzymes of the epidermis of human skin, 149
  effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid on skin lipid synthesis (^{14}C incorporation) *in vitro*, 608
  lichen planus; an inborn error of metabolism, 341
  lipid synthesis in rat skin, 586
  measurement of lipid synthesis in mouse auricular skin cultured *in vitro*, 475
Book reviews:
  Consultations in Dermatology with Walter B. Shelley, 290
  Current Concepts of the Histology of Oral Mucosa, ed. C. A. Squier and J. Meyer, 86
  Current Problems in Dermatology, Vol. 4 ed. J. W. H. Mali, 83
  Cutaneous Lesions of the Lower Extremities, M. H. Samitz and A. S. Dana, 179
  Dermatology Specialty Board Review, V. J. Selmanowitz and N. Orentreich, 391
  Hazards of Medication. A Manual of Interactions, Incompatibilities, Contraindications, and Adverse Effects, ed. E. W. Martin and R. D. Martin, 290
  Mémento Immunologique de la Sarcoidose, J. Chretien and J. C. Saltiel, 84
  Protection Against Radiation from Brachytherapy Sources, NCRP Publications, Report No. 40, 515
  Radium Recipes for Cutaneous Cancer (The Manchester Method), J. B. Howell, W. J. Meredith and G. Swindell, 391

Regulation of Mast Cell Formation, ed. G. Csaba, 179
The Pathologic Anatomy of Mycoses, ed. R. D. Baker, 84
The Third Conference on the Clinical Delineation of Birth Defects, Part XII. Skin, Hair and Nails, ed. D. Bergsma, 291
Bowen's disease of the vulva: review of recent literature on, 165

Candida albicans; ultrastructural features of the invasive phase of, 435
— infections of the vulva: review of the recent literature on, 64
Carbamezepine; cutaneous reactions to, 647
Carcinogenesis of the vulva: review of recent literature on, 163
Carcinoma, basal cell; of the vulva: review of recent literature on, 164
— of bronchus, associated with epidermolysis bullosa (case), 70
— —, associated with porphyria cutanea tarda, rheumatoid arthritis and right hydronephrosis (case title), 289
—, squamous (epidermoid); of the vulva: review of recent literature on, 165
Cells: cell life span (post-mitotic) of epidermis, 189
epidermal cells *in vitro*; effects of cytochalasin B on, 580
human epidermal cell cycle *in vitro*, 13
lymphocytes and nickel antibodies, 320
—; deficient folate activity during treatment of psoriasis with methotrexate, diagnosed by determination of serine synthesis in, 248
polymorphonuclear leukocytes; chronic repetitive suppuration of the skin associated with a serum factor affecting the bactericidal capacity of (case title), 289
Cellular immunity, altered; in pyoderma gangrenosum, 529
Chalone mechanism and cancer, 352
— — system of epidermis, 188
Chancroid of the vulva: review of the recent literature on, 63
Cheyletiella yasguri Smiley; dermatitis in man and in a dog caused by, 245
Chlamydiae infections of the vulva: review of recent literature on, 59
Chloracne (case title), 289
Chloroquine therapy, low dose, and the action of chloroquine in symptomatic porphyria; studies on, 261
CIBA 1906 (thiambutosine BP) in the treatment of vitiligo (letter), 81
Circulation; role of in measurements of skin conductivity, 213
Clindamycin; effect of in acne—a clinical and laboratory investigation, 37
Comment, 53, 161, 280, 386, 509
Complement in pemphigus; direct immunofluorescent study of the skin on occurrence of, 466
Compositae, liverworts and lichens; immunological specificity of, 235
Contact Dermatitis XIX, 400
Copper and zinc in psoriasis (letter), 291
Corrections, 403, 404, 522
Correspondence, 73, 176, 291, 392, 516
Crohn's disease of the mouth, 501
Cutis laxa; physical properties of skin in (letter), 176
Cyclic 3′,5′ adenosine monophosphate-dependent protein kinase of skin.
I. Measurement and properties, 571
II. Levels in atopic dermatitis and psoriasis, 577
Cytochalasin B; effects of on epidermal cells *in vitro*, 580
Cytology of mycosis fungoides and the Sézary syndrome, 96

Deafness, physical retardation and peripheral neuropathy; atypical erythroderma associated with, 308
Degos' syndrome (malignant atrophic papulosis) (case), 394
Dermatitis, atopic; levels of cyclic 3′,5′ adenosine monophosphate-dependent protein kinase of skin in, 577
—, chronic superficial, 405
—, contact: allergic contact dermatitis to garlic, 6
— — — to sesquiterpenoids of plants, 235
chromate sensitivity in cement dermatitis, 400
necrotic reactions to applied medicaments, 401
photocontact dermatitis to the dye, Disperse Blue 35 (letter), 392
— in man and in a dog caused by the mite *Cheyletiella yasguri* Smiley, 245
—, industrial; prognosis in, 145
—, perioral, 430
—, —: aetiology and treatment with tetracycline, 351
—, seborrhoeic; of the beard, 241
"Dermographism, delayed" and actinic reticuloid (case), 396
Dermonecrotic factor in pyoderma gangrenosum, 529
Detergents, enzyme; effect of on transepidermal water loss from normal skin, 10
Disperse Blue 35; photocontact dermatitis to (letter), 392
Drug Reactions XIX, 292; XX, 646
Dyslipidosis, Anderson-Fabry (case title), 398

Eicosa 5 : 8 : 11 : 14 tetraynoic acid; effect of on skin lipid synthesis (^{14}C incorporation) *in vitro*, 608
Elastic fibres, cutaneous, in lupus erythematosus; ultrastructural study of, 355
Electron microscopy: abnormal hair shafts in psoriasis on scanning electron microscopy, 368
  cytology of mycosis fungoides and the Sézary syndrome. Correlation between light and electron microscopy, 96
  electron microscopy: a functional approach, 53
  studies in the inflammatory reaction in pityriasis lichenoides, 534
  study of 6 cases of corticosteroid-induced atrophy of the skin with telangiectasia, 548
  study of hypopigmented lesions in leprosy, 200
  ultrastructural features of the invasive phase of *Candida albicans*, 435
  ultrastructural study of the cutaneous elastic fibres in lupus erythematosus, 355
  ultrastructure of lichen amyloidosus with special reference to the epidermal changes, 117
Enzymes: cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin.
  I. Measurement and properties, 571
  II. Levels in atopic dermatitis and psoriasis, 577
  glucose-6-phosphate dehydrogenase in normal human skin and skin from lichen planus patients, 341
  metabolic enzymes of the epidermis of human skin; effect of chronic sun damage on the activity of, 149
Eosinophilia, pulmonary; associated with vasculitis and extra-vascular granulomata, 412
Epidermal cell cycle (human) *in vitro*, 13
— —s *in vitro*; effects of cytochalasin B on, 580
— chalone system, 188
Epidermis; control of thickness of, 187, 347
—, cow snout; changes in the fatty acid composition of the phospholipids, triglycerides and free fatty acids with depth in, 227
—, human: acid hydrolases in, 450
—, —; effect of chronic sun damage on the activity of metabolic enzymes of, 149
—; prostaglandins and, 161
Epidermolysis bullosa associated with neoplasm of the bronchus (case), 70
Epilepsy, pemphigoid and gross hyperkeratosis of the palms and soles (case title), 174
Epithelioma cuniculatum (case), 71
Erythrasma lesions; a quantitative study of 130
Erythroderma due to peritrate and glyceryl trinitrate, 498
Erythrokeratoderma, atypical; with deafness, physical retardation and peripheral neuropathy, 308
European Society for Dermatological Research and the Society for Investigative Dermatology; report of joint meeting, Amsterdam, May 1972, 398

Fatty acid composition of the phospholipids, triglycerides and free fatty acids; changes in with depth in the cow snout epidermis, 227
Fibrinogen, I^{125} labelled, in deep venous thrombosis, 518
Folate activity, deficient, during treatment of psoriasis with methotrexate, diagnosed by determination of serine synthesis in lymphocytes, 248
Free fatty acids, phospholipids and triglycerides; changes in the fatty acid composition of with depth in the cow snout epidermis, 227
Fungal infections: *Candida albicans;* ultrastructural features of the invasive phase of, 435
  Candida infections of the vulva: review of recent literature on, 64
  tinea nigra; a case of, in Britain, 505
  Trichophyton mentagrophytes ringworm, generalized, in Capuchin monkeys (Cebus nigrivitatus), 565

Garlic; allergic contact dermatitis due to, 6
Glands of Montgomery (letter), 177
Glucose tolerance and insulin production in patients with granuloma annulare, 540
Glucose-6-phosphate dehydrogenase in normal human skin and skin from lichen planus patients, 341
Glycerol; effect of on the percutaneous absorption of methyl nicotinate, 30
Glyceryl trinitrate and peritrate; erythroderma due to, 498
Granuloma annulare; insulin production and glucose tolerance in patients with, 540
— inguinale of the vulva: review of the recent literature on, 63
"—, wheat-stubble sarcoid": a new epithelioid granuloma of the skin, 444
Granulomata, extra-vascular, and vasculitis; pulmonary eosinophilia associated with, 412
Griseofulvin therapy in lichen planus, 383

Hair diameter in female baldness, 361

"— extension", causing traction alopecia (letter), 79
— replacement in the guinea-pig and in man, 509
— shafts, abnormal, in psoriasis, on scanning electron microscopy, 368
Herpesvirus hominis infections of the vulva: review of recent literature on, 62
Histochemistry: pityriasis lichenoides; epidermal component of, 107
Homeostasis; epidermal, 197
Hydantoin derivatives; cutaneous reactions to, 646
Hydroa vacciniforme (case), 395
Hydrolases, acid, in the human epidermis, 450
Hydronephrosis, carcinoma of bronchus, rheumatoid arthritis; porphyria cutanea tarda in association with (case title), 289
Hyperkeratosis, gross, of palms and soles; pemphigoid and epilepsy (case title), 174
Hypopigmented lesions in leprosy; electron microscopic study of, 200

Immunology: cell-mediated immunity in patients with malignant melanoma, 523
  direct immunofluorescent study of the skin on occurrence of complement in pemphigus, 466
  immunofluorescent studies in the inflammatory reaction in pityriasis lichenoides, 534
  pyoderma gangrenosum with altered cellular immunity and dermonecrotic factor, 529
Impetigo contagiosa; aetiology of, 420
Innervation of sweat glands in horses and dogs (letter), 80
Insulin production and glucose tolerance in patients with granuloma annulare, 540
Irish Dermatological Society, 514
Irradiation, cobalt; followed by localized acneiform eruption, 274
Isoniazid and streptomycin in the treatment of tuberculosis cutis, 270

Jejunal biopsies in bullous pemphigoid (letter), 177

Lactose intolerance; acrodermatitis enteropathica with, 174
Leishmaniasis, post kala-azar dermal, 374
Leprosy; electron microscopic study of hypopigmented lesions in, 200
Leukocytes, polymorphonuclear; chronic repetitive suppuration of the skin associated with a serum factor affecting the bactericidal capacity of (case title), 289
Lichen amyloidosis, familial, 123
— —; ultrastructure of, with special reference to the epidermal changes, 117
— myxoedematosus (case), 172
— planus; an inborn error of metabolism, 341
— —; griseofulvin therapy in, 383
— —; pathogenesis of (letter), 81
Lichens, compositae and liverworts; immunological specificity of, 235
Lipid lowering agents, 180
— synthesis in mouse auricular skin cultured *in vitro*; measurement of, 475
— — in rat skin, 586
— — in skin (^{14}C incorporation) *in vitro*; effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid on, 608
Liverworts, compositae and lichens; immunological specificity of, 235
Lupus erythematosus, discoid; an unusual presentation of, 642
— —, —; in association with fatty infiltration of liver (alcoholic) and ?porphyria (case title), 289
— —; ultrastructural study of the cutaneous elastic fibres in, 355
Lymphocyte transformation and nickel antibodies, 320
—s; deficient folate activity during treatment of psoriasis with methotrexate diagnosed by determination of serine synthesis in, 248
Lymphocytoma cutis (case), 288
Lymphoma; "giant" orf lesion on the finger occurring in a patient with (case title), 398

Malignancy, internal; Senear-Usher syndrome and, 1
Mastocytosis, massive nodular; congenital macular urticaria pigmentosa complicated by, with systemic involvement, 635
Melanoma, Fortner's amelanotic hamster AMel 3; combined surgical and chemotherapeutic treatment of, 18
—, malignant; cell-mediated immunity in patients with, 523
—, —; of the vulva: review of recent literature on, 164
Methotrexate; deficient folate activity during treatment of psoriasis with, diagnosed by determination of serine synthesis in lymphocytes, 248
— therapy; structural and functional abnormalities of the liver in psoriasis before and during, 623
Methyl nicotinate; effect of glycerol on the percutaneous absorption of, 30
Mitotic activity of epidermis; range of, 191
Montgomery; glands of (letter), 177
Mycoplasma infections of the vulva: review of recent literature on, 59
Mycosis fungoides and parapsoriasis en plaques, 405

— — and the Sézary syndrome; cytology of, 96
— — presenting as a solitary tumour (case), 514

Naevi, unilateral spider; pregnancy associated with, 51
Naevus lipomatosus cutaneus superficialis (Hoffman-Zurhelle), 557
2-Naphthol; percutaneous absorption and distribution of in man, 614
Neuropathy (peripheral), deafness and physical retardation; atypical erythrokeratoderma associated with, 308
News and Notices, 186, 297, 402, 522
Nickel antibodies, 320

Obituary:
  Davidson, Andrew McC., 390
  Franklin, John L., 284
  Henly, Gilbert, 286
  Ingram, John T., 512
  MacGregor, Kenneth, 72
  Ritter, Eric C., 285
  Ritterman, Gedeon H., 287
Oral-facial-digital syndrome (case), 171
Orf lesion, "giant", on the finger occurring in a patient with a lymphoma (case title), 398
Organ culture; action of staphylococcal epidermolytic toxin on mouse skin in, 516
Oxazolidine derivatives, cutaneous reactions to, 647

Paget's disease of the vulva; review of recent literature on, 164
Papillomata, eruptive basal cell, with underlying mitotic lesion (case title), 72
Papulosis, malignant atrophic (Degos' syndrome) (case), 394
Parapsoriasis en plaques (chronic superficial dermatitis) and pre-reticulotic poikiloderma; natural history of, 405
Patch test reactions in man, skin allergic and irritant; comparative study of ^{3}H-thymidine labelling of the dermal infiltrate of, 460
Pediculosis, vulvar: review of recent literature on, 64
Pemphigoid, epilepsy and gross hyperkeratosis of the palms and soles (case title), 174
—, bullous; jejunal biopsies in (letter), 177
Pemphigus as an autoimmune disease (letter), 78
—; direct immunofluorescent study of the skin on occurrence of complement in, 466
Penicillins: adverse cutaneous reactions to, 293
Percutaneous absorption and distribution of 2-naphthol in man, 614
— — of methyl nicotinate; effect of glycerol on, 30
Periodicals:
  American Zoologist, 515
  Arbeitsmedizin, Sozialmedizin, Arbeitshygiene, 515
Peritrate and glyceryl trinitrate; erythroderma due to, 498
Phlebography, 518
Phospholipids, triglycerides and free fatty acids; changes in the fatty acid composition of with depth in the cow snout epidermis, 227
Pilosebaceous duct obstruction and acne, 327
Pityriasis lichenoides and toxoplasmosis, 114
— —; epidermal component of, 106
— —; the inflammatory reaction in, 533
— rubra pilaris; treatment of with azathioprine, 42
Poikiloderma atrophicans vasculare, 408
—, pre-reticulotic, and parapsoriasis en plaques (chronic superficial dermatitis); natural history of, 405
Polyarteritis nodosa, cutaneous, 87
?Porphyria, and fatty infiltration of liver (alcoholic); discoid lupus erythematosus in association with (case title), 289
— cutanea tarda, carcinoma of bronchus, rheumatoid arthritis, right hydronephrosis (case title), 289
— — — (Proceedings of the International Symposium, Rome 1970), 174
—, symptomatic; studies on low dose chloroquine therapy and the action of chloroquine in, 261
Prostaglandins and the epidermis, 161
Psoriasis; abnormal hair shafts in, on scanning electron microscopy, 368
—; alopecia in (letter), 73
—; deficient folate activity during treatment of with methotrexate diagnosed by determination of serine synthesis in lymphocytes, 248
—; effect of retinoic acid in—II, 256
—; levels of cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin in, 577
—; retinoic acid in the treatment of (letter), 392
—; structural and functional abnormalities of the liver in, before and during methotrexate therapy, 623
—; ultraviolet therapy of, 379
—; zinc and copper in (letter), 291
Pyoderma gangrenosum with altered cellular immunity and dermonecrotic factor, 529

Retardation (physical), deafness and peripheral neuropathy; atypical erythrokeratoderma associated with, 308
Reticuloid, actinic (case), 397
—, —, and " delayed dermographism " (case), 396
Retinoic acid; effect of in psoriasis—II, 256
— — in the treatment of psoriasis (letter), 392
Rheumatoid arthritis, carcinoma of bronchus, right hydronephrosis; porphyria cutanea tarda in association with (case title), 289
Rosacea-like eruption of children, 425
Royal Society of Medicine, 70, 171, 288, 394

Sarcoma of the vulva: review of recent literature on, 163
Scabies, unusual features of, associated with topical fluorinated steroids, 496
Schistosomiasis of the vulva: review of the recent literature on, 64
Sebum excretion rate and skin surface lipid composition; age and sex variation in, 333
Senear-Usher syndrome and internal malignancy, 1
Serine synthesis in lymphocytes; deficient folate activity during treatment of psoriasis with methotrexate diagnosed by determination of, 248
Sesquiterpene lactones, allergenic, 235
Sesquiterpenoids of plants; allergic contact dermatitis from, 235
Sézary syndrome and mycosis fungoides; cytology of, 96
Skin conductivity; role of the circulation in measurements of, 213
Society for Investigative Dermatology and the European Society for Dermatological Research; report of joint meeting, Amsterdam, May 1972, 398
Steroids, topical fluorinated; unusual features of scabies associated with, 496
Stratum corneum; separation of (letter), 516
Strawberry mark; earliest features of, in the newborn, 138
Streptomycin and isoniazid in the treatment of tuberculosis cutis, 270
Succinimide derivatives, cutaneous reactions to, 647
Sun damage, chronic; effect of on the activity of metabolic enzymes of the epidermis of human skin, 149
Suppuration of the skin, chronic repetitive; associated with a serum factor affecting the bactericidal capacity of polymorphonuclear leukocytes (case title), 289
Surface lipid of skin, composition of, and sebum excretion rate; age and sex variation in, 333
Sweat glands in horses and dogs; innervation of (letter), 80
Sweating; conditioning of, 154

Telangiectasia, in association with corticosteroid-induced atrophy of the skin, 548
Tetracycline in the treatment of perioral dermatitis, 351
Tetraynoic acid (eicosa 5 : 8 : 11 : 14); effect of on skin lipid synthesis (^{14}C incorporation) *in vitro*, 608
Therapeutics XIX, 180; XX, 518
Thiambutosine BP (CIBA 1906) in the treatment of vitiligo (letter), 81
Thrombosis, deep venous; management of, 518
^{3}H-Thymidine labelling of the dermal infiltrate of skin allergic and irritant patch test reactions in man; comparative study of, 460
Tinea nigra in Britain; a case of, 505
Topical applications; recent progress in the formulation of vehicles for, 386
Toxin, staphylococcal epidermolytic; action of on mouse skin in organ culture (letter), 516
Toxoplasmosis and pityriasis lichenoides, 114
Transepidermal water loss from normal skin; effect of enzyme detergents on, 10
Treatment: azathioprine in pityriasis rubra pilaris, 42
  chloroquine in symptomatic porphyria, 261
  CIBA 1906 (thiambutosine BP) in vitiligo (letter), 81
  clindamycin in acne, 37
  combined surgical and chemotherapeutic treatment of Fortner's amelanotic hamster melanoma AMel 3, 18
  griseofulvin in lichen planus, 383
  methotrexate in psoriasis; deficient folate activity during treatment, diagnosed by determination of serine synthesis in lymphocytes, 248
  — — —; structural and functional abnormalities of the liver before and during therapy, 623
  retinoic acid in psoriasis—II, 256; (letter), 392
  streptomycin and isoniazid in tuberculosis cutis, 270
  tetracycline in perioral dermatitis, 351
  ultraviolet light in psoriasis, 379
  zinc sulphate in chronic venous leg ulceration; double-blind trial of, 632
Trichomonas infections of the vulva: review of recent literature on, 64
Trichophyton mentagrophytes ringworm, generalized; in Capuchin monkeys (*Cebus nigrivitatus*), 565
Triglycerides, phospholipids and free fatty acids; changes in the fatty acid composition of with depth in the cow snout epidermis, 227

Tuberculosis cutis; intermittent chemotherapy in the treatment of, 270
— of the vulva: review of recent literature on, 63
Tumours of the vulva: review of recent literature on, 163

Ulcer, benign " malignant ", 46
Ulceration of leg, chronic venous; double-blind trial of zinc sulphate in the treatment of, 632
Ultrasound in deep venous thrombosis, 518
Ultraviolet therapy in psoriasis, 379
Urticaria pigmentosa, congenital macular, complicated by massive nodular mastocytosis with systemic involvement, 635

Vasculitis and extra-vascular granulomata; pulmonary eosinophilia associated with, 412
—, hereditary inflammatory, with persistent nodules, 299
—, single component cryoglobulinaemic (case title), 396
Vehicles for topical applications; recent progress in the formulation of, 386
Vincaleucoblastine in treatment of Fortner's amelanotic hamster melanoma AMel 3, 18
Virus infections of the vulva: review of recent literature on, 59
Vitiligo treated with CIBA 1906 (thiambutosine BP) (letter), 81
Vulva, diseases of the; review of the recent literature on—Part II. Vulvitis: infections, 58. Part III. Carcinogenesis and tumours, 163
Vulvitis: infections. Review of recent literature on, 58

Waardenburg's syndrome in two siblings, both their parents and their maternal grandmother (case title), 174
Warts, vulvar: review of recent literature on, 60

Xeroderma pigmentosum (case title), 72

Zinc and copper in psoriasis (letter), 291
— sulphate in the treatment of chronic venous leg ulceration; double-blind trial of, 632

AUTHORS

Abraham, G. J. S., Malik, Gauri B.; Sehgal, V. N. and: griseofulvin therapy in lichen planus, 383

Achten, G., de Bast, Cl.; Delescluse, J. and: pyoderma gangrenosum with altered cellular immunity and dermonecrotic factor, 529

Adams, Rowan, Hales, C. L. and Raffle, E. J., the effect of enzyme detergents on transepidermal water loss from normal skin, 10

Alexander, Suzanne; Forman, L. and: nickel antibodies, 320

—, Marks, R.; Savin, J. A. and: a rosacea-like eruption of children, 425

Allen, Ingrid V., Nevin, N. C., Froggatt, P., Kernohan, D. C.; Beare, J. M. and: atypical erythrokeratoderma with deafness, physical retardation and peripheral neuropathy, 308

Almeyda, J., epidermolysis bullosa associated with neoplasm of the bronchus (case), 70

—, Barnardo, D., Baker, H., Levene, G. M. and Landells, J. W., structural and functional abnormalities of the liver in psoriasis before and during methotrexate therapy, 623

— and Levantine, A., Drug Reactions XIX, 293; XX, 646

Andreev, V. C.; Zlatkov, N. B. and: toxoplasmosis and pityriasis lichenoides, 114

Arndt, K. A.; Parrish, J. A. and: seborrhoeic dermatitis of the beard, 241

Aziz, A. A., Soliman, M.; El Zawahry, M. and: the aetiology of impetigo contagiosa, 420

Bagnall, B. G. and Grünberg, W., generalized *Trichophyton mentagrophytes* ringworm in Capuchin monkeys (*Cebus nigrivitatus*), 565

Baker, H., Barnardo, D., Levene, G. M., Landells, J. W.; Almeyda, J. and: structural and functional abnormalities of the liver in psoriasis before and during methotrexate therapy, 623

Bakkers, E. J. M. and Fain, A.; dermatitis in man and in a dog caused by the mite *Cheyletiella yasguri* Smiley, 245

Bakos, L., Storck, R. and Netto, E. S., congenital macular urticaria pigmentosa complicated by massive nodular mastocytosis with systemic involvement, 635

Barnardo, D., Baker, H., Levene, G. M., Landells, J. W.; Almeyda, J. and: structural and functional abnormalities of the liver in psoriasis before and during methotrexate therapy, 623

Beare, J. M., Nevin, N. C., Froggatt, P., Kernohan, D. C. and Allen, Ingrid V., atypical erythrokeratoderma with deafness, physical retardation and peripheral neuropathy, 308

Beighton, P.; Grahame, R. and: the physical properties of skin in cutis laxa (letter), 176

Bergeron, R. F., Tuffanelli, D., Wilson Jones, E.; Reed, W. B. and: hereditary inflammatory vasculitis with persistent nodules, 299

Bettley, F. R., the effect of a medicated wash on acne (letter), 292

—, Ramsay, C.; Cream, J. J. and: single component cryoglobulinaemic vasculitis (case title), 396

Black, M. M., the nature, pathogenesis and staining properties of amyloid, 280

— and Heather, C. J., ultrastructure of lichen amyloidosus with special reference to the epidermal changes, 117

—; Marks, R. and: the epidermal component of pityriasis lichenoides, 106

—, —, the inflammatory reaction in pityriasis lichenoides, 533

—; Savage, J. and: " giant " orf lesion on the finger occurring in a patient with a lymphoma (case title), 398

Bleumink, E., Doeglas, H. M. G., Klokke, A. H. and Nater, J. P., allergic contact dermatitis to garlic, 6

Borrie, P., cutaneous polyarteritis nodosa, 87

Bottoms, Eva, Comaish, S.; Wyatt, E. and: abnormal hair shafts in psoriasis on scanning electron microscopy, 368

Bullough, W. S., the control of epidermal thickness, 187, 347

Bulusu, L., Cunliffe, W. J., Williamson, B.; Cotterill, J. A. and: age and sex variation in skin surface lipid composition and sebum excretion rate, 333

Burrows, D., prognosis in industrial dermatitis, 145

Burton, J. L. and Burton, P. A., pulmonary eosinophilia associated with vasculitis and extra-vascular granulomata, 412

Butterworth, M. J., Dodman, B.; Cunliffe, W. J. and: unilateral spider naevi, 51

Cairns, R. J., oral-facial-digital syndrome (case), 171

Campbell, C. K.; Chadfield, H. W. and: a case of tinea nigra in Britain, 505

Carney, S. A., Hall, M. and Ricketts, C. R., the adenosine triphosphate content of guinea-pig skin stored at −196°C, 23

Castro, A., Dyess, K.; Hammond, R. and: insulin production and glucose tolerance in patients with granuloma annulare, 540

Cawson, R. A. and Rajasingham, K. C., ultrastructural features of the invasive phase of *Candida albicans*, 435

Cerimele, D., Yamasawa, S. and Serri, F., the effect of chronic sun damage on the activity of metabolic enzymes of the epidermis of human skin, 149

Chadfield, H. W. and Campbell, C. K., a case of tinea nigra in Britain, 505

Chopra, E. P. and Flaxman, B. A., human epidermal cell cycle *in vitro*, 13

Chorzelski, T. and Jabłońska, S., pemphigus as an autoimmune disease (letter), 78

Church, R. E., Ebling, F. J.; Jackson, D. and: hair diameter in female baldness, 361

—; Jensen, N. E. and: malignant atrophic papulosis (Degos' syndrome) (case), 394

Cochran, A. J., Spilg, W. G. S., Thomas, C. E.; MacKie, Rona M. and: cell-mediated immunity in patients with malignant melanoma, 523

Comaish, S., Bottoms, Eva; Wyatt, E. and: abnormal hair shafts in psoriasis on scanning electron microscopy, 368

Constable, Hilary, Cruickshank, C. N. D. and Mann, Patricia R., effects of cytochalasin B on epidermal cells *in vitro*, 580

Cormane, R. H., Pondman, K. W.; van Joost, Th. and: direct immunofluorescent study of the skin on occurrence of complement in pemphigus, 466

Cotterill, J. A., Williamson, B.; Cunliffe, W. J. and: the effect of clindamycin in acne—a clinical and laboratory investigation, 37

—, —, — and Bulusu, L., age and sex variation in skin surface lipid composition and sebum excretion rate, 333

Cotton, D. W. K. and Hasselt, P. v., innervation of sweat glands in horses and dogs (letter), 80

—; Kuypers, B. R. M. and: conditioning of sweating, 154

—, Van den Hurk, J. J. M. A. and Van der Staak, W. B. J. M., lichen planus; an inborn error of metabolism, 341

Cream, J. J., Bettley, F. R. and Ramsay, C., single component cryoglobulinaemic vasculitis (case title), 396

Cronin, E.; Wilkinson, D. S. and: Contact Dermatitis XIX, 400

Cruickshank, C. N. D., Mann, Patricia R.; Constable, Hilary, and: effects of cytochalasin B on epidermal cells *in vitro*, 580

Cunliffe, W. J., Cotterill, J. A. and Williamson, B., the effect of clindamycin in acne—a clinical and laboratory investigation, 37

—, Dodman, B. and Butterworth, M. J., unilateral spider naevi, 51

—, Williams, M.; Holmes, R. L. and: pilo-sebaceous duct obstruction and acne, 327

—, Williamson, B., Bulusu, L.; Cotterill, J. A. and: age and sex variation in skin surface lipid composition and sebum excretion rate, 333

David, T. J. and Warin, R. P., Waardenburg's syndrome in two siblings, both their parents and their maternal grandmother (case title), 174

De Bast, Cl., Achten, G.; Delescluse, J. and: pyoderma gangrenosum with altered cellular immunity and dermonecrotic factor, 529

De Matteis, F., porphyria cutanea tarda (Proceedings of the International Symposium, Rome 1970), 174

Delescluse, J., De Bast, Cl and Achten, G., pyoderma gangrenosum with altered cellular immunity and dermonecrotic factor, 529

Deppe, W. M., Shanley, B. C., Stewart-Wynne, E. G., Joubert, S. M.; Taljaard, J. J. F. and: studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria, 261

Diaz-Flores, L., Sánchez Yus, E., Simon Huarte, P.; Gomez Orbaneja, J. and: cytology of mycosis fungoides and the Sézary syndrome. Correlation between light and electron microscopy, 96

Dodman, B., Butterworth, M. J.; Cunliffe, W. J. and: unilateral spider naevi, 51

Doeglas, H. M. G., Klokke, A. H., Nater, J. P.; Bleumink, E. and: allergic contact dermatitis to garlic, 6

Du Vivier, A., Jopling, W. H.; Munro, D. D. and: post kala-azar dermal leishmaniasis, 374

—; Munro, D. D. and: lymphocytoma cutis (case), 288

Dupuis, G., Geissman, T. A.; Mitchell J. C. and: allergic contact dermatitis from sesquiterpenoids of plants. Additional allergenic sesquiterpene lactones and immunological specificity of compositae, liverworts and lichens, 235

Dyess, K., Castro, A.; Hammond, R. and: insulin production and glucose tolerance in patients with granuloma annulare, 540

Eady, R. A. J., Moynahan, E. J. and Wilkinson, J. F., gross hyperkeratosis of the palms and soles, pemphigoid and epilepsy (case title), 174

Ebling, F. J., Church, R. E.; Jackson, D. and: hair diameter in female baldness, 361

Edwards, I. R., Walker, Anne E.; Frenk, E. and: Therapeutics XIX, 180; XX, 518

El Zawahry, M., Aziz, A. A. and Soliman, M., the aetiology of impetigo contagiosa, 420

Ellegaard, J., Esmann, V. and Henriksen, L.; deficient folate activity during treatment of psoriasis with methotrexate diagnosed by determination of serine synthesis in lymphocytes, 248

Esmann, V., Henriksen, L.; Ellegaard, J. and: deficient folate activity during treatment of psoriasis with methotrexate diagnosed by determination of serine synthesis in lymphocytes, 248

Ewen, S. W. B., Kerr, N. W.; Stankler, L. and: Crohn's disease of the mouth, 501

Fain, A.; Bakkers, E. J. M. and: dermatitis in man and in a dog caused by the mite *Cheyletiella yasguri* Smiley, 245

Feiwel, M.; Macdonald, A. and: discoid lupus erythematosus, fatty infiltration of liver (alcoholic) and ?porphyria (case title), 289

—; —: perioral dermatitis: aetiology and treatment with tetracycline, 351

—; Nyman, C. R. and: porphyria cutanea tarda, carcinoma of bronchus, rheumatoid arthritis, right hydronephrosis (case title), 289

Ferguson, T. F. M., Hartop, P. J.; Prottey, C. and: lipid synthesis in rat skin, 586

—; Prottey, C. and: measurement of lipid synthesis in mouse auricular skin cultured *in vitro*, 475

Finley, A. G. and Musso, L. A., naevus lipomatosus cutaneus superficialis (Hoffman-Zurhelle), 557

Flaxman, B. A.; Chopra, E. P. and: human epidermal cell cycle *in vitro*, 13

Fleming, K. A.; Haroon, T. S. and: an unusual presentation of discoid lupus erythematosus, 642

Forbes, I. J.; Hunter, G. A. and: treatment of pityriasis rubra pilaris with azathioprine, 42

Forman, L. and Alexander, Suzanne; nickel antibodies, 320

Frenk, E., Walker, Anne E. and Edwards, I. R., Therapeutics XIX, 180; XX, 518

Froggatt, P., Kernohan, D. C., Nevin, N. C., Allen, Ingrid V.; Beare, J. M. and: atypical erythrokeratoderma with deafness, physical retardation and peripheral neuropathy, 308

Fry, L., McMinn, R. M. H.; Macdonald, A. and: effect of retinoic acid in psoriasis—II, 256

Gafoor, A. and Townsend, K. M. S., separation of stratum corneum (letter), 516

Gaylarde, P. M.; Sarkany, I. and: action of staphylococcal epidermolytic toxin on mouse skin in organ culture (letter), 516

—; —: the pathogenesis of lichen planus (letter), 81

Geissman, T. A., Dupuis, G.; Mitchell, J. C. and: allergic contact dermatitis from sesquiterpenoids of plants. Additional allergenic sesquiterpene lactones and immunological specificity of compositae, liverworts and lichens, 235

Giannelli, F., Wallace, H. J.; Kobza, A. and: xeroderma pigmentosum (case title), 72

Gilkes, J. J. H. and Wells, R. S., lichen myxoedematosus (case), 172

Goh, Y. S.; Rajan, V. S. and: intermittent chemotherapy in the treatment of tuberculosis cutis, 270

Goldschmidt, H., Leyden, J. J.; Stein, K. M. and: localized acneiform eruption following cobalt irradiation, 274

Gomez Orbaneja, J., Sánchez Yus, E., Diaz-Flores, L. and Simon Huarte, P., cytology of mycosis fungoides and the Sézary syndrome. Correlation between light and electron microscopy, 96

Grahame, R. and Beighton, P., the physical properties of skin in cutis laxa (letter), 176

Greaves, M. W., prostaglandins and the epidermis, 161

— and Ive, F. A., double-blind trial of zinc sulphate in the treatment of chronic venous leg ulceration, 632

Grünberg, W.; Bagnall, B. G. and: generalized *Trichophyton mentagrophytes* ringworm in Capuchin monkeys (*Cebus nigrivitatus*), 565

Hadgraft, J., Hadgraft, J. W. and Sarkany, I., the effect of glycerol on the percutaneous absorption of methyl nicotinate, 30

Hadgraft, J. W., recent progress in the formulation of vehicles for topical applications, 386

Hales, C. L., Raffle, E. J.; Adams, Rowan and: the effect of enzyme detergents on transepidermal water loss from normal skin, 10

Hall, M., Ricketts, C. R.; Carney, S. A. and: the adenosine triphosphate content of guinea-pig skin stored at −196°C, 23

Hall-Smith, P.; Ramsay, C. and: hydroa vacciniforme (case), 395

Halprin, K. M., Taylor, J. R., Levine, V.; Ohkawara, A. and: acid hydrolases in the human epidermis, 450

Hammond R., Dyess, K. and Castro, A., insulin production and glucose tolerance in patients with granuloma annulare, 540

Haneke, E., Wohlrab, W. and Peker, J., combined surgical and chemotherapeutic treatment of Fortner's amelanotic hamster melanoma AMel 3, 18

Harman, R. R. M., traction alopecia due to " hair extension " (letter), 79

Haroon, T. S. and Fleming, K. A., an unusual presentation of discoid lupus erythematosus, 642

Hartop, P. J., Ferguson, T. F. M.; Prottey, C. and: lipid synthesis in rat skin, 586

Heather, C. J.; Black, M. M. and: ultrastructure of lichen amyloidosus with special reference to the epidermal changes, 117

Hemels, H. G. W. M., percutaneous absorption and distribution of 2-naphthol in man, 614

Henriksen, L., Esmann, V.; Ellegaard, J. and: deficient folate activity during treatment of psoriasis with methotrexate diagnosed by determination of serine synthesis in lymphocytes, 248

Herman, P. S., photocontact dermatitis in a dye manufacturing process (letter), 392

Hidano, A. and Nakajima, S., earliest features of the strawberry mark in the newborn, 138

Hodgson, G. A. and Thompson, Eileen; chronic repetitive suppuration of the skin associated with a serum factor affecting the bactericidal capacity of polymorphonuclear leukocytes (case title), 289

Holla, S. W. J., Van den Hurk, J., Hollman, E. P. M. J., Porters, J. E., Weemers, M. B. M.; Mier P. D. and: cyclic 3′ 5′-adenosine monophosphate-dependent protein kinase of skin. II. Levels in atopic dermatitis and psoriasis, 577

Hollman, E. P. M. J., Van den Hurk, J., Holla, S. W. J., Porters, J. E., Weemers, M. B. M.; Mier, P. D. and: cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. II. Levels in atopic dermatitis and psoriasis, 577

Holmes, R. L., Williams, M. and Cunliffe, W. J., pilo-sebaceous duct obstruction and acne, 327

Hunter, G. A. and Forbes, I. J., treatment of pityriasis rubra pilaris with azathioprine, 42

Hunter, J. A. A., electron microscopy: a functional approach, 53

Ive, F. A.; Greaves, M. W. and: double-blind trial of zinc sulphate in the treatment of chronic venous leg ulceration, 632

Jabłońska, S.; Chorzelski, T. and: pemphigus as an autoimmune disease (letter), 78

Jackson, D., hair replacement in the guinea-pig and in man, 509

—, Church, R. E. and Ebling, F. J., hair diameter in female baldness, 361

Jensen, N. E. and Church, R. E., malignant atrophic papulosis (Degos' syndrome) (case), 394

—, Sneddon, I. B. and Walker, Anne E., chloracne: three cases (case title), 289

Job, C. K., Nayar, Ammu and Narayanan, J. S., electronmicroscopic study of hypopigmented lesions in leprosy, 200

Johnson, M. L., Anderson-Fabry dyslipidosis (case title), 398

Jopling, W. H., Du Vivier, A.; Munro, D. D. and: post kala-azar dermal leishmaniasis, 374

Joubert, S. M., Shanley, B. C., Stewart-Wynne, E. G., Deppe, W. M.; Taljaard, J. J. F. and: studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria, 261

Kernohan, D. C., Nevin, N. C., Froggatt, P., Allen, Ingrid V.; Beare, J. M. and: atypical erythrokeratoderma with deafness, physical retardation and peripheral neuropathy, 308

Kerr, N. W., Ewen, S. W. B.; Stankler, L. and: Crohn's disease of the mouth, 501

Klokke, A. H., Doeglas, H. M. G., Nater, J. P.; Bleumink, E. and: allergic contact dermatitis to garlic, 6

Kobza, A., Giannelli, F. and Wallace, H. J., xeroderma pigmentosum (case title), 72

Kuypers, B. R. M. and Cotton, D. W. K., conditioning of sweating, 154

Lachapelle, J. M., comparative study of ^{3}H-thymidine labelling of the dermal infiltrate of skin allergic and irritant patch test reactions in man, 460

Landells, J. W., Barnardo, D., Baker, H., Levene, G. M.: Almeyda, J. and: structural and functional abnormalities of the liver in psoriasis before and during methotrexate therapy, 623

Levantine, A.; Almeyda, J. and: Drug Reactions, XIX, 293; XX, 646

Levene, G. M., Barnardo, D., Baker, H., Landells, J. W.; Almeyda, J. and: structural and functional abnormalities of the liver in psoriasis before and during methotrexate therapy, 623

Levine, V., Halprin, K. M., Taylor, J. R.; Ohkawara, A. and: acid hydrolases in the human epidermis, 450

Lewis, J., Robinson, T. W. E.; Walter, J. A. and: eruptive basal cell papillomata with underlying mitotic lesion (case title), 72

Leyden, J. J., Goldschmidt, H.; Stein, K. M. and: localized acneiform eruption following cobalt irradiation, 274

Long, V. J. W., changes in the fatty acid composition of the phospholipids, triglycerides and free fatty acids with depth in the cow snout epidermis, 227

MacConnell, Linda E. S.; Saikia, N. K. and: Senear-Usher syndrome and internal malignancy, 1

Macdonald, A. and Feiwel, M., discoid lupus erythematosus, fatty infiltration of liver (alcoholic) and ?porphyria (case title), 289

—, —, perioral dermatitis: aetiology and treatment with tetracycline, 351

—, McMinn, R. M. H. and Fry, L., effect of retinoic acid in psoriasis—II, 256

MacKie, Rona, McQueen, A.; Saikia, N. K. and: jejunal biopsies in bullous pemphigoid (letter), 177

—, Spilg, W. G. S., Thomas, C. E. and Cochran, A. J., cell-mediated immunity in patients with malignant melanoma, 523

Macmillan, A. L., unusual features of scabies associated with topical fluorinated steroids, 496

McMinn, R. M. H., Fry, L.; Macdonald, A. and: effect of retinoic acid in psoriasis—II, 256

McQueen, A., MacKie, Rona; Saikia, N. K. and: jejunal biopsies in bullous pemphigoid (letter), 177

Malik, Gauri B., Abraham, G. J. S.; Sehgal, V. N. and: griseofulvin therapy in lichen planus, 383

Mann, Patricia R., Cruickshank, C. N. D.; Constable, Hilary, and: effects of cytochalasin B on epidermal cells *in vitro*, 580

Marks, R., Alexander, Suzanne; Savin, J. A. and: a rosacea-like eruption of children, 425

— and Black, M. M., the epidermal component of pityriasis lichenoides, 106

—; —, the inflammatory reaction in pityriasis lichenoides, 533

Meenan, F. O. C., the glands of Montgomery (letter), 177

Mier, P. D. and Van den Hurk, J., cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. I. Measurement and properties, 571

—, —, Holla, S. W. J., Hollman, E. P. M. J., Porters, J. E. and Weemers, M. B. M., cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. II. Levels in atopic dermatitis and psoriasis, 577

Milla, P. J. and Moynahan, E. J., acrodermatitis enteropathica with lactose intolerance (case title), 174

Mitchell, D., mycosis fungoides presenting as a solitary tumour (case), 514

Mitchell, J. C., Dupuis, G. and Geissman, T. A., allergic contact dermatitis from sesquiterpenoids of plants. Additional allergenic sesquiterpene lactones and immunological specificity of compositae, liverworts and lichens, 235

Molokhia, M.; Portnoy, B. and: zinc and copper in psoriasis (letter), 290

Moynahan, E. J.; Milla, P. J. and: acrodermatitis enteropathica with lactose intolerance (case title), 174

—, Wilkinson, J. F.; Eady, R. A. J. and: gross hyperkeratosis of the palms and soles, pemphigoid and epilepsy (case title), 174

Munro, D. D. and Du Vivier, A., lymphocytoma cutis (case), 288

—, — and Jopling, W. H., post kala-azar dermal leishmaniasis, 374

Musso, L. A.; Finley, A. G. and: naevus lipomatosus cutaneus superficialis (Hoffman-Zurhelle), 557

Nakajima, S.; Hidano, A. and: earliest features of the strawberry mark in the newborn, 138

Narayanan, J. S., Nayar, Ammu; Job, C. K. and: electronmicroscopic study of hypopigmented lesions in leprosy, 200

Nater, J. P., Doeglas, H. M. G., Klokke, A. H.; Bleumink, E. and: allergic contact dermatitis to garlic, 6

Nayar, Ammu, Narayanan, J. S.; Job, C. K. and: electronmicroscopic study of hypopigmented lesions in leprosy, 200

Netto, E. S., Storck, R.; Bakos, L. and: congenital macular urticaria pigmentosa complicated by massive nodular mastocytosis with systemic involvement, 635

Nevin, N. C., Froggatt, P., Kernohan, D. C., Allen, Ingrid V.; Beare, J. M. and: atypical erythrokeratoderma with deafness, physical retardation and peripheral neuropathy, 308

Newbold, P. C. H. and Stoughton, R. B., a benign " malignant " ulcer, 46

Nyman, C. R. and Feiwel, M., porphyria cutanea tarda, carcinoma of bronchus, rheumatoid arthritis, right hydronephrosis (case title), 289

Ohkawara, A., Halprin, K. M., Taylor, J. R. and Levine, V., acid hydrolases in the human epidermis, 450

Parrish, J. A. and Arndt, K. A., seborrhoeic dermatitis of the beard, 241

Peker, J., Wohlrab, W.; Haneke, E. and: combined surgical and chemotherapeutic treatment of Fortner's amelanotic hamster melanoma AMel 3, 18

Perrot, H., Thivolet, J.; Schmitt, D. and: ultrastructural study of the cutaneous elastic fibres in lupus erythematosus, 355

Pimentel, J. C., the " wheat-stubble sarcoid granuloma": a new epithelioid granuloma of the skin, 444

Pondman, K. W., Cormane, R. H.; van Joost, Th. and: direct immunofluorescent study of the skin on occurrence of complement in pemphigus, 466

Porters, J. E., Van den Hurk, J., Holla, S. W. J., Hollman, E. P. M. J., Weemers, M. B. M.; Mier, P. D. and: cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. II. Levels in atopic dermatitis and psoriasis 577

Portnoy, B. and Molokhia, M., zinc and copper in psoriasis (letter), 291

Prottey, C. and Ferguson, T. F. M., measurement of lipid synthesis in mouse auricular skin cultured *in vitro*, 475

—, Hartop, P. J. and Ferguson, T. F. M., lipid synthesis in rat skin, 586

Raffle, E. J., Hales, C. L.; Adams, Rowan, and: the effect of enzyme detergents on transepidermal water loss from normal skin, 10

Rajagopalan, K. and Tay, C. H., familial lichen amyloidosis, 123

Rajan, V. S. and Goh, Y. S., intermittent chemotherapy in the treatment of tuberculosis cutis, 270

Rajasingham, K. C.; Cawson, R. A. and: ultrastructural features of the invasive phase of *Candida albicans*, 435

Ramsay, C., Bettley, F. R.; Cream, J. J. and: single component cryoglobulinaemic vasculitis (case title), 396

— and Hall-Smith, P., hydroa vacciniforme (case), 395

— and Scott, O. L. S., actinic reticuloid and " delayed dermographism " (case), 396

Reed, W. B., Bergeron, R. F., Tuffanelli, D. and Wilson Jones, E., hereditary inflammatory vasculitis with persistent nodules, 299

Ricketts, C. R., Hall, M.; Carney, S. A. and: the adenosine triphosphate content of guinea-pig skin stored at −196°C, 23

Ridley, C. M., a review of the recent literature on diseases of the vulva. Part II—Vulvitis: infections, 58. Part III—Carcinogenesis and tumours, 163

Robinson, T. W. E., Lewis, J.; Walter, J. A. and: eruptive basal cell papillomata with underlying mitotic lesion (case title), 72

Ryan, F. P., erythroderma due to peritrate and glyceryl trinitrate, 498

Saikia, N. K. and MacConnell, Linda E. S., Senear-Usher syndrome and internal malignancy, 1

—, McQueen, A. and MacKie, Rona, jejunal biopsies in bullous pemphigoid (letter), 177

Samman, P. D., the natural history of parapsoriasis en plaques (chronic superficial dermatitis) and pre-reticulotic poikiloderma, 405

Sánchez Yus, E., Diaz-Flores, L., Simon Huarte, P.; Gomez Orbaneja, J. and: cytology of mycosis fungoides and the Sézary syndrome. Correlation between light and electron microscopy, 96

Sarkany, I. and Gaylarde, P. M., action of staphylococcal epidermolytic toxin on mouse skin in organ culture (letter), 516

—, —: the pathogenesis of lichen planus (letter), 81

—, Hadgraft, J. W.; Hadgraft, J. and: the effect of glycerol on the percutaneous absorption of methyl nicotinate, 30

Savage, J. and Black, M. M., " giant " orf lesion on the finger occurring in a patient with a lymphoma (case title), 398

Savin, J. A., Alexander, Suzanne and Marks, R., a rosacea-like eruption of children, 425

Schmitt, D., Thivolet, J. and Perrot, H., ultrastructural study of the cutaneous elastic fibres in lupus erythematosus, 355

Scott, O. L. S.; Ramsay, C. and: actinic reticuloid and " delayed dermographism " (case), 396

Sehgal, V. N., Abraham, G. J. S. and Malik, Gauri B., griseofulvin therapy in lichen planus, 383

Serri, F., Yamasawa, S.; Cerimele, D. and: the effect of chronic sun damage on the activity of metabolic enzymes of the epidermis of human skin, 149

Shanley, B. C., Stewart-Wynne, E. G., Deppe, W. M., Joubert, S. M.; Taljaard, J. J. F. and: studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria, 261

Shuster, S., psoriatic alopecia (letter), 73

Simon Huarte, P., Sánchez Yus, E., Diaz-Flores, L.; Gomez Orbaneja, J. and: cytology of mycosis fungoides and the Sézary syndrome. Correlation between light and electron microscopy, 96

Sneddon, I., perioral dermatitis, 430

—, Walker, Anne E.; Jensen, N. E. and: chloracne: three cases (case title), 289

Soliman, M., Aziz, A. A.; El Zawahry, M. and: the aetiology of impetigo contagiosa, 420

Somerville, Dorothy A., a quantitative study of erythrasma lesions, 130

Spilg, W. G. S., Thomas, C. E., Cochran, A. J.; MacKie, Rona M. and: cell-mediated immunity in patients with malignant melanoma, 523

Stankler, L., Ewen, S. W. B. and Kerr, N. W., Crohn's disease of the mouth, 501

Stein, K. M., Leyden, J. J. and Goldschmidt, H., localized acneiform eruption following cobalt irradiation, 274

Stevanović, D. V., corticosteroid-induced atrophy of the skin with telangiectasia, 548

Stewart-Wynne, E. G., Shanley, B. C., Deppe, W. M., Joubert, S. M.; Taljaard, J. J. F. and: studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria, 261

Storck, R., Netto, E. S.; Bakos, L. and: congenital macular urticaria pigmentosa complicated by massive nodular mastocytosis with systemic involvement, 635

Stoughton, R. B.; Newbold, P. C. H. and: a benign " malignant " ulcer, 46

Summerly, R., Woodbury, Sheila and Yardley, H. J., the effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid on skin lipid synthesis (^{14}C incorporation) *in vitro*, 608

Taljaard, J. J. F., Shanley, B. C., Stewart-Wynne, E. G., Deppe, W. M. and Joubert, S. M., studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria, 261

Tay, C. H.; Rajagopalan, K. and: familial lichen amyloidosis, 123

Taylor, J. R., Halprin, K. M., Levine, V.; Ohkawara, A. and: acid hydrolases in the human epidermis, 450

Thivolet, J., Perrot, H.; Schmitt, D. and: ultrastructural study of the cutaneous elastic fibres in lupus erythematosus, 355

Thomas, C. E., Spilg, W. G. S., Cochran, A. J.; MacKie, Rona M. and: cell-mediated immunity in patients with malignant melanoma, 523

Thompson, Eileen; Hodgson, G. A. and: chronic repetitive suppuration of the skin associated with a serum factor affecting the bactericidal capacity of polymorphonuclear leukocytes (case title), 289

Thorne, N., epithelioma cuniculatum (case), 71

Townsend, K. M. S.; Gafoor, A. and: separation of stratum corneum (letter), 516

Tuffanelli, D., Bergeron, R. F., Wilson Jones, E.; Reed, W. B. and: hereditary inflammatory vasculitis with persistent nodules, 299

Van den Hurk, J., Holla, S. W. J., Hollman, E. P. M. J., Porters, J. E., Weemers, M. B. M.; Mier, P. D. and: cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. II. Levels in atopic dermatitis and psoriasis, 577

—; Mier, P. D. and: cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. I. Measurement and properties, 571

—, Van der Staak, W. B. J. M.; Cotton, D. W. K. and: lichen planus; an inborn error of metabolism, 341

Van der Staak. W. N. J. M., Van den Hurk, J. J. M. A.; Cotton, D. W. K. and: lichen planus; an inborn error of metabolism, 341

Van Hasselt, P.; Cotton, D. W. K. and: innervation of sweat glands in horses and dogs (letter), 80

Van Joost, Th., Cormane, R. H. and Pondman, K. W., direct immunofluorescent study of the skin on occurrence of complement in pemphigus, 466

Verbov, J., use of CIBA, 1906 (thiambutosine BP) in vitiligo (letter), 81

Walker, Anne E., Edwards, I. R.; Frenk, E. and: Therapeutics XIX, 180; XX, 518

—, Sneddon, I. B.; Jensen, N. E. and: chloracne: three cases (case title), 289

Wallace, H. J., Giannelli, F.; Kobza, A. and: xeroderma pigmentosum (case title), 72

Walter, J. A., Lewis, J. and Robinson, T. W. E., eruptive basal cell papillomata with underlying mitotic lesion (case title), 72

Warin, R. P.; David, T. J. and: Waardenburg's syndrome in two siblings, both their parents and their maternal grandmother (case title), 174

Weemers, M. B. M., Van den Hurk, J., Holla, S. W. J., Hollman, E. P. M. J., Porters, J. E.; Mier, P. D. and: cyclic 3′,5′-adenosine monophosphate-dependent protein kinase of skin. II. Levels in atopic dermatitis and psoriasis, 577

Wells, R. S.; Gilkes, J. J. H. and: lichen myxoedematosus (case), 172

Whittle C. H., retinoic acid in the treatment of psoriasis (letter), 392

Williams, M., Cunliffe, W. J.; Holmes, R. L. and: pilo-sebaceous duct obstruction and acne, 327

Williamson, B., Cotterill, J. A.; Cunliffe, W. J. and: the effect of clindamycin in acne—a clinical and laboratory investigation, 37

—, Cunliffe, W. J., Bulusu, L.; Cotterill, J. A. and: age and sex variation in skin surface lipid composition and sebum excretion rate, 333

Wilkinson, D. S., actinic reticuloid (case), 397

— and Cronin, E., Contact Dermatitis XIX, 400

Wilkinson, J. F., Moynahan, E. J.; Eady, R. A. J. and: gross hyperkeratosis of the palms and soles, pemphigoid and epilepsy (case title), 174

Wilson Jones, E., Bergeron, R. F., Tuffanelli, D.; Reed, W. B. and: hereditary inflammatory vasculitis with persistent nodules, 299

Wohlrab, W., Peker, J.; Haneke, E. and: combined surgical and chemotherapeutic treatment of Fortner's amelanotic hamster melanoma AMel 3, 18

Woodbury, Sheila, Yardley, H. J.; Summerly, R. and: the effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid on skin lipid synthesis (^{14}C incorporation) *in vitro*, 608

Woolley-Hart, Ann: the role of the circulation in measurements of skin conductivity, 213

Wyatt, E., Bottoms, Eva and Comaish, S., abnormal hair shafts in psoriasis on scanning electron microscopy, 368

Yamasawa, S., Serri, F.; Cerimele, D. and: the effect of chronic sun damage on the activity of metabolic enzymes of the epidermis of human skin, 149

Yardley, H. J., Woodbury, Sheila; Summerly, R. and: the effect of eicosa 5 : 8 : 11 : 14 tetraynoic acid on skin lipid synthesis (^{14}C incorporation) *in vitro*, 608

Young, E., ultraviolet therapy of psoriasis: a critical study, 379

Zlatkov, N. B. and Andreev, V. C., toxoplasmosis and pityriasis lichenoides, 114

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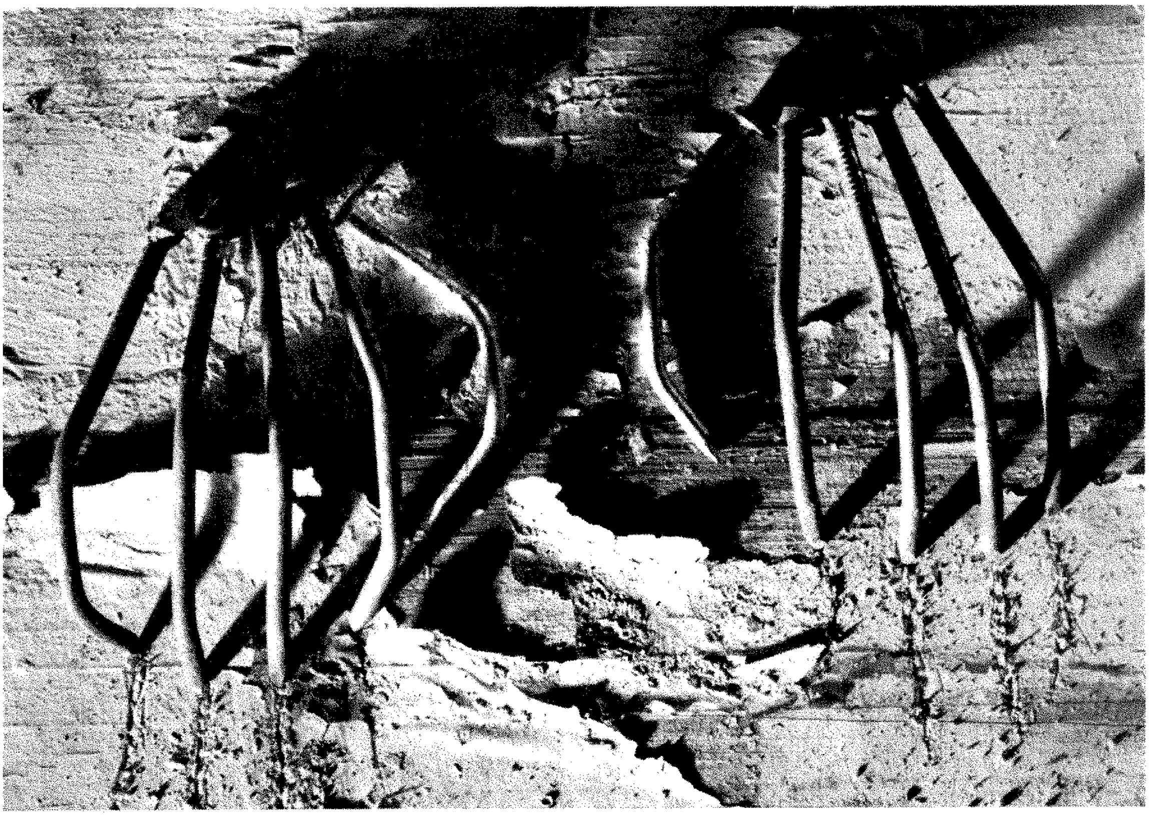

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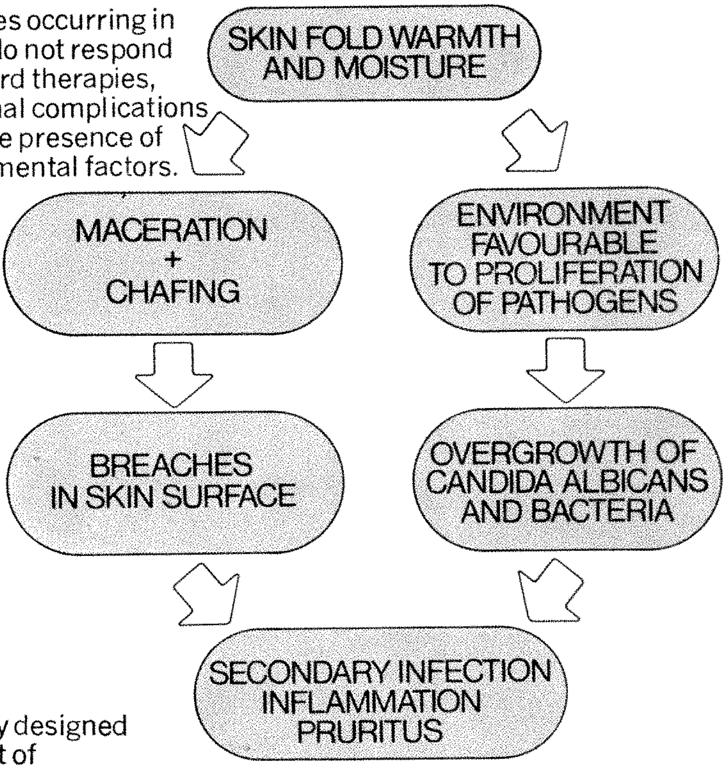
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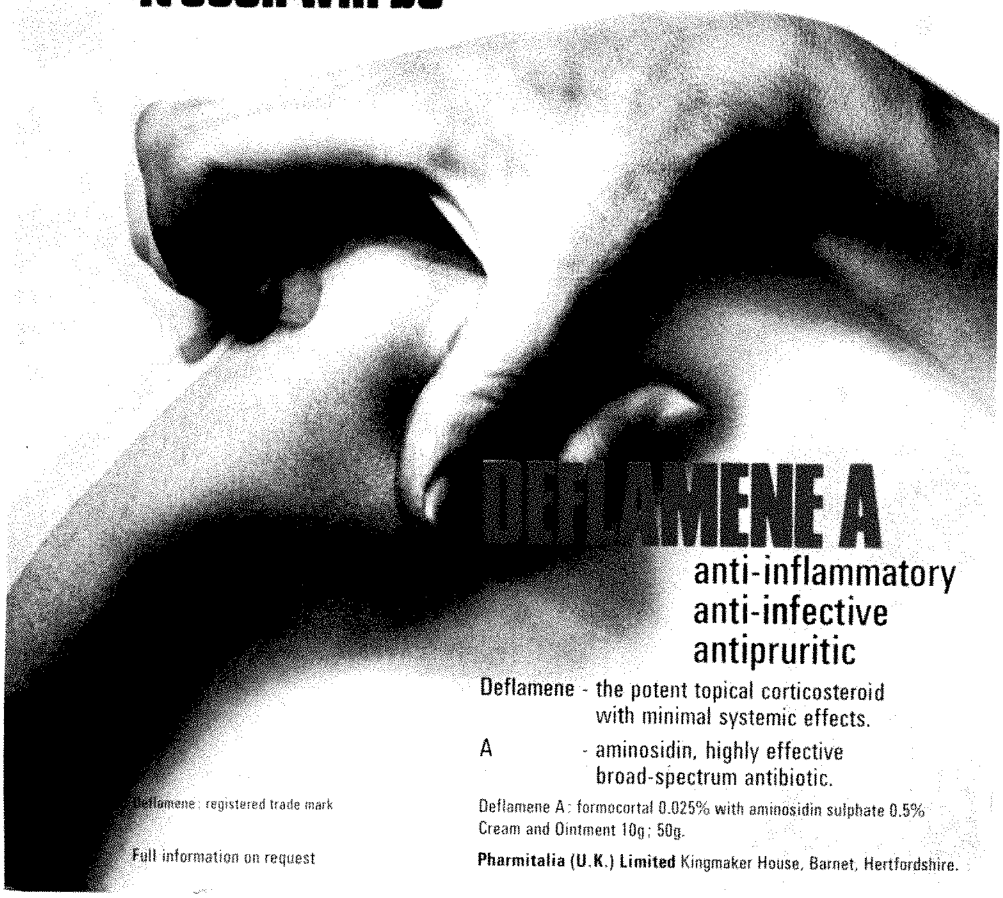

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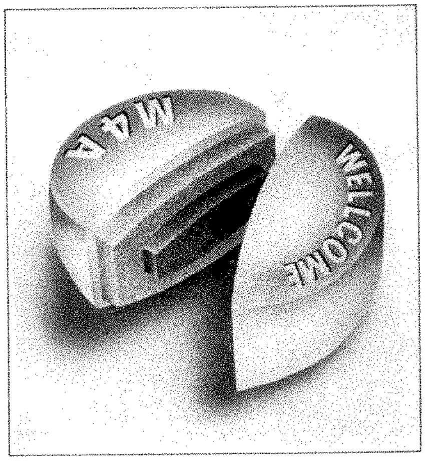

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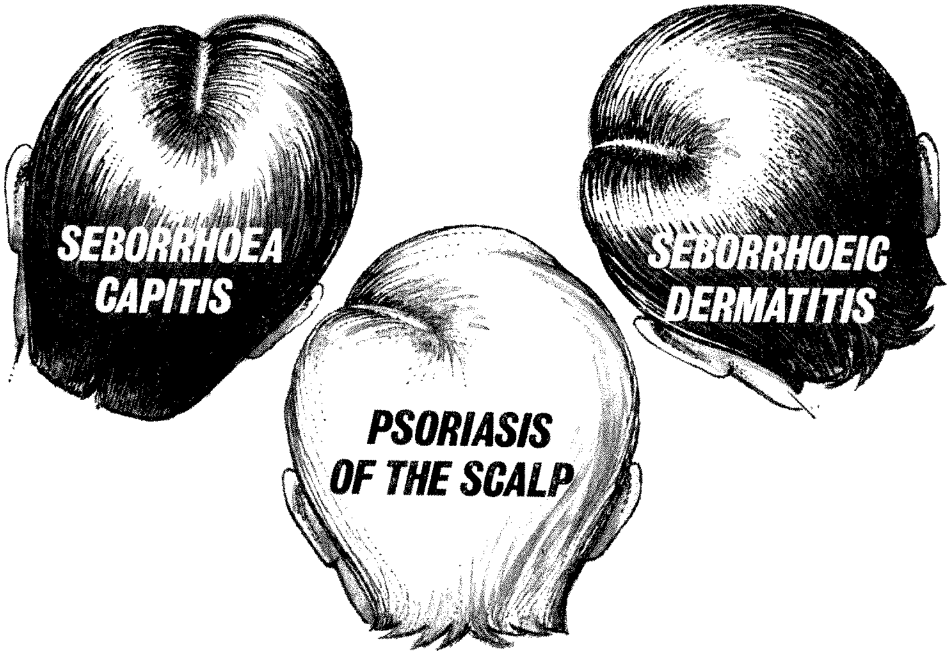
Burroughs Wellcome & Co. (The Wellcome Foundation Ltd.) Berkhamsted, Herts.



CEANEL CONCENTRATE

A DEVELOPMENT BY QUINODERM

Ceanel is an extremely versatile scalp and hair cleaner. Its versatility makes it an effective Descaling and Decrusting Agent. It can also be used for cleaning other areas of the skin. For free samples and literature please write to :
Quinoderm Ltd., Freepost, Oldham OL1 2YY
(no postage needed).

Formulae: Phenylethyl Alcohol B.P.C. 7.5. Cetrimide B.P. 10.0. Undecenoic Acid B.P.C. 1.0. Lanolin in a desquamative base.

QUINODERM LIMITED, OLDHAM, ENGLAND

Prioderm

(0·5% Malathion Lotion)

the *surest* control of head infestation

"Nearly 3,000 people have been treated with 0.5% malathion lotion (PRIODERM) and no live lice have been recovered from any case on the following day. The ovicidal effect is complete and the residual effect considerable".

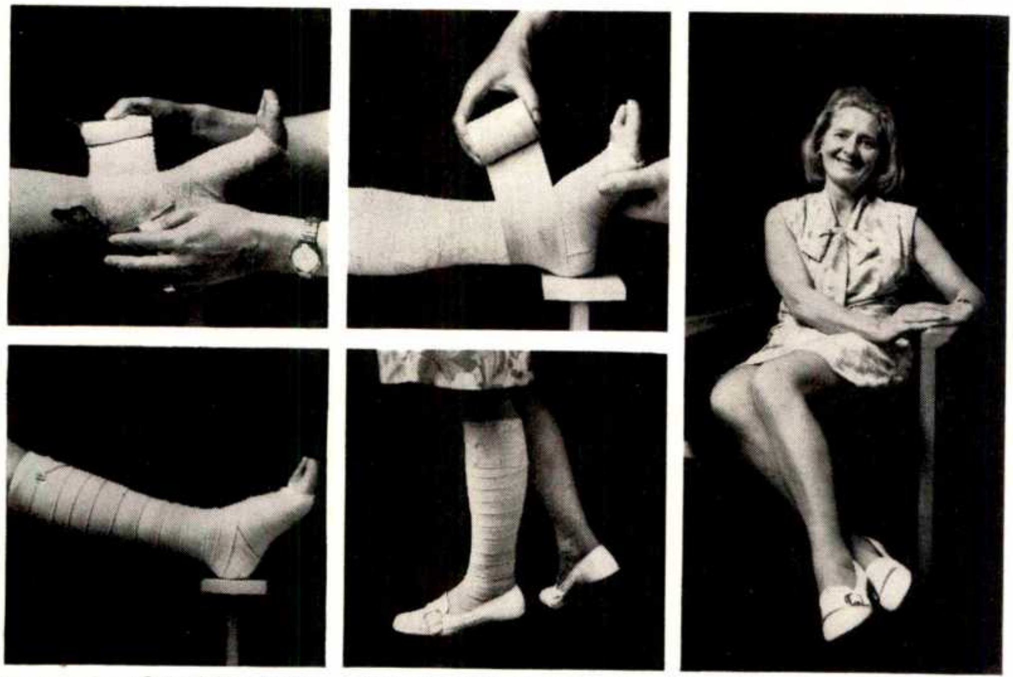
"We have the technical abilities needed to virtually exterminate the louse. What is needed now is an end to half-hearted measures".

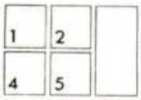
Community Medicine (1971) 126.145

Packing: Bottle 55 ml. Full information is available from:

Priory Laboratories Limited West Drayton Middx.

The effective treatment of leg ulcers...by Seton





Stage 1: Prepare the leg and apply Seton Medicated Paste Bandages.
Stage 2: Apply Seton Lestreflex Diachylon Bandage.
Stage 3: Repeat Stages 1 and 2 until ulcer is healed.
Stage 4: Remove Bandages and apply Seton Varico Pressure Bandage.
Stage 5: Encourage patient to exercise leg to assist and maintain recovery.

Very briefly, that is Seton's recommended procedure, based on twenty years of continuous research and experience, in co-operation with medical authorities. A new booklet showing medicated and compression bandage techniques for treating ulceration and similar ailments of the lower limbs, is available free on request.

The products which Seton manufacture specifically for these treatments are:

Seton Medicated Paste Bandages:
7.5 cm × 6 m.
Zincaband (Zinc Paste/BPC)
Icthaband (Zinc Paste and Ichthammol/BPC)
Uraband (Zinc Paste with Ichthammol and Urethane/Drug Tariff)
Calaband (Zinc Paste with Calamine and Urethane/Drug Tariff)
Tarband (Zinc Paste and Coal Tar/BPC)
Quinaband (Zinc Paste with Iodochlorohydroxyquinoline/ Drug Tariff)

Lestreflex
elastic diachylon bandages, plain or ventilated.
7.5 cm × 4.5 m stretched. (BPC)

Blue Line elastic web bandages with central blue line.
7.5 cm width. Available in lengths of 3m, 5m, 10m, and 25m. (Drug Tariff)

Varico pressure bandage, made from blue-line material, complete with foot loop to assist application by patient.
7.5 cm × 2.25 m (unstitched portion).
(Drug Tariff)

Seton
Products Limited
Tubiton House, Medlock Street,
Oldham OL1 3HS.
Telephone: 061-652 2222 (5 lines)

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British Journal of Dermatology

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CONTENTS (*continued*)

Case Reports

Congenital Macular Urticaria Pigmentosa Complicated by Massive Nodular Mastocytosis with Systemic Involvement
Lucio Bakos, Ronildo Storck and Ernesto Silveira Netto . . 635
An Unusual Presentation of Discoid Lupus Erythematosus
T. S. Haroon and K. A. Fleming 642

Quarterly Review

Drug Reactions XX. Cutaneous Reactions to Anticonvulsants
John Almeyda and Ashley Levantine 646

Index Volume 87 651